Remarks

Claims 1, 3, 5-7, 9-12, 20, and 22 are pending in this application; claims 7, 9, and 10 are withdrawn. With this reply, Applicants have amended claims 1 and 3 and added new claims 23 and 24, which are generic to the elected species, "lymphoma." Accordingly, upon entry of this amendment, claims 1, 3, 5, 6, 11, 12, and 22-24 are under examination.

Claim 1 has been amended to write out the terms "Thomsen-Friedenreich antigen" and "mucin 1," as requested by the Examiner. Claim 3 has been amended to recite "which express on the cell surface TF, MUC1, and glycophorin." These amendments are supported by the application as-filed, for example, on page 4 of the specification. Support for new claims 23 and 24 can be found, for example, on page 50, lines 19-23 and page 51, line 25 to page 52, line 5. These amendments do not add new matter.

Information Disclosure Statement

The Examiner indicated that two references listed on previously-filed Information Disclosure Statements, Ichiyama, *Kareiigaku Kenkyusho Zasshi* 51(3,4): 93-110 (2000) and Goletz et al., *Adv. Exp. Med. Biol.* 535:147-62 (2003), were not considered. These two references, however, were cited in the current Office Action in rejections under 35 U.S.C. § 102(b) and 35 U.S.C. § 112, first paragraph (enablement), respectively. Accordingly, Applicants understand these documents to have been considered by the Examiner.

Claim Objections

The Examiner has objected to claims 1, 11, and 20, stating that the abbreviations TF and MUC1 should be spelled out at their first appearance in the claims. Applicants have amended claim 1 as the Examiner suggested, obviating the objection.

Rejections under 35 U.S.C. § 112, first paragraph, enablement

Claim 3: Deposit Declaration

The Examiner has rejected claim 3 as allegedly not enabled. Specifically, the Examiner alleges that the cell lines NM-F9 and NM-D4 recited in claim 3 do not comply with the deposit Rules for biological materials. Applicants enclose with this paper a Deposit Declaration signed by an authorized agent of assignee Glycotope GmbH, which indicates that the recited strains were deposited pursuant to the requirement of 37 C.F.R §§ 1.801-1.1809. Accordingly, the rejection should be withdrawn.

Claims 20 and 22

The Examiner also alleges that claims 20 and 22 are not enabled. The Examiner states that the specification "does not provide enablement for claims directed to methods of treating or preventing lymphoma in a subject by administering a cell line expressing TF, MUC1 and glycophorin on its surface as broadly claimed." The Examiner acknowledges that the application provides data indicating induction of T helper immune responses and memory immune responses against MUC1, TF, and AGPA in NOD/SCID mice reconstituted with human PMBC vaccinated with NM-F9 cell lysates. The specification teaches that NM-F9 cells express TF, MUC1, and glycophorin on their surface. The Examiner states, however, that the specification is silent about *in vivo* administration of a cell line which expresses on the cell surface TF,

MUC1 and glycophorin to treat or prevent lymphoma. Office Action at 9. The Examiner states that "the instant issue is whether or not the prior art and the as-filed application provides [sic] sufficient guidance and the degrees [sic] of predictability as to the structural and functional correlation between the administration of a cell line expressing TF, MUC1 and glycophorin on its surface to achieve a therapeutic effect in the treatment or prevention of lymphoma." *Id.* at 11. The Examiner cites two publications to suggest that different types of lymphoma require specific therapies. The Examiner concludes that empirical testing would be required for each different type of lymphoma and equates such empirical testing with undue experimentation. *Id.* at 10-11. The Examiner does not identify any subject matter believed to be enabled. Applicants respectfully traverse.

The M.P.E.P. reiterates the standard articulated by the Federal Circuit for determining compliance with the enablement requirement of 35 U.S.C. § 112, first paragraph: "[t]he test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without *undue* experimentation." M.P.E.P.§ 2164.01 (emphasis added), quoting *United States v. Telectronics, Inc.,* 8 USPQ2d 1217, 1223 (Fed. Cir. 1988). The Examiner bears the initial burden to establish a reasonable basis to question enablement, which must be supported by specific technical reasoning. *See, e.g.,* M.P.E.P. §§ 2164.01 and 2164.04; *see also In re Marzocchi,* 169 USPQ 367, 370 (CCPA 1971)("it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain *why* it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning

which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure."). Evidence supporting enablement, in turn, "need not be *conclusive* but merely *convincing* to one skilled in the art." M.P.E.P. § 2164.05, emphasis in original. Moreover, the Examiner should always attempt to identify enabled subject matter. *See, e.g.*, M.P.E.P. §§ 2164.04 and 2164.08.

Applicants respectfully submit that the Examiner has not met the initial burden of providing specific reasoning to overcome the presumption that Applicants' claims are enabled. The Examiner merely generalizes that different lymphomas require different therapeutics, empirical testing is needed to apply any particular therapeutic to each type of lymphoma, and such testing constitutes undue experimentation. The rejection does not specifically address Applicants' teaching that cells expressing TF, MUC1, and glycophorin, which are known to be expressed on tumor cells, can be used to raise immune responses *in vitro* and *in vivo* in a model of the human immune system. The Examiner essentially dismisses these teachings because they do not explicitly demonstrate treating lymphoma *in vivo*. However, a skilled artisan would believe that Applicants' results reasonably correlate with the claimed methods of treatment because the art recognizes that these antigens are expressed on a wide array of cancers, including lymphoma, and immunotherapy is an accepted approach to treating cancer. Thus, there is a reasonable expectation that Applicants' claimed methods will be effective for treating cancer, including lymphoma.

The application provides extensive guidance for how to make and use the cells of the invention and a clear experimental template for a person having ordinary skill in the

art to use the cells to elicit an immune response to one or more of MUC1, TF, and glycophorin—as well as *in vivo* data showing that lysates of the cells successfully induce an immune response to all three antigens in a model of the *human* immune system. Adjusting the dose of cells to accommodate a human subject, for example, is well within the skill of the art. Applicants have provided adequate guidance to enable the skilled artisan to practice the claimed methods, which the skilled artisan would believe correlate with the Examples in the application, meeting the requirements of 35 U.S.C. § 112, first paragraph.

The Office Action refers to several of the factors endorsed by the Federal Circuit in *In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988), for helping to determine whether experimentation may be undue: the breadth of the claims, knowledge and level of predictability in the art, amount of direction provided by the inventor, existence of working examples, and quantity of experimentation. Applicants address these issues in turn, below.

At the outset, however, Applicants note that the Examiner's emphasis on the lack of an example conclusively demonstrating *in vivo* efficacy of the claimed methods is misplaced. "Compliance with the enablement requirement," however, "does not turn on whether an example is disclosed." M.P.E.P. § 2164.02. Requiring Applicants to demonstrate *in vivo* efficacy against lymphoma is unduly burdensome and contradicts the instruction from the courts and the M.P.E.P. that only "a *reasonable* correlation between the activity in question and the asserted utility" is needed. M.P.E.P. § 2107.03(I); *see also* M.P.E.P. § 2164.02 ("the [E]xaminer must also give reasons for

a conclusion of lack of correlation for an *in vitro* or *in vivo* animal model example [in an enablement rejection]").

l. Quantity of experimentation/claim scope

The Examiner alleges that each lymphoma to be treated by the methods of the invention must be empirically tested and that the quantity of experimentation required includes de novo determination of effective target sites, modes of delivery, safe administration of the cells recited in the claims to target appropriate cells and/or tissues in any lymphoma in a mammal, including a human. Office Action at 11. Thus, the Examiner appears to be arguing that a large amount of experimentation is needed to practice the claims in their current scope.

Applicants first note that, with respect to issues of safety of a particular treatment, other government agencies are responsible for ensuring conformance with safety standards, and "[t]he Office must confine its review of patent applications to the statutory requirements of the patent law." M.P.E.P. § 2107.03(V). Regarding the quantity of experimentation, the Federal Circuit and M.P.E.P. recognize that "a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." M.P.E.P. § 2164.06, quoting *Wands*, 8 USPQ2d at 1404 (citing *In re Angstadt* 190 USPQ 214, 217-19 (CCPA 1976)).

There is no question that the application shows how to make cells that express the pan-carcinoma cell surface antigens TF, MUC1, and glycophorin, as well as how to use lysates of those cells to produce an immune response to these antigens *in vivo*.

See, e.g., Examples 1 and 4, respectively. Moreover, Example 5 shows that these cells are hypersensitive to cell lysis by NK cells, supporting the inference that the immune response elicited by cell lysates could be replicated by administering the cells directly. Adapting these teachings to a particular mode of delivery and fine-tuning dosage to achieve an immune response in a particular patient is well within the ordinary skill in the art. In fact, pages 24-39 of the specification describe some of the ways an artisan can do so. *See also* Freireich *et al.*, *Cancer Chemother. Rep.* 50:219-244 (1966) (describing how to convert dosages between different organisms, including mouse and human).

Regarding targeting the claimed treatment to appropriate cells or tissues, the power of the present invention is, in part, that it recruits a patient's own immune system to seek out tumor cells that express one or more of the pan-carcinomic markers TF, glycophorin, and MUC1. Accordingly, contrary to the Examiner's concern, little or no experimentation is needed to target the therapy to the desired cells or tissues, obviating the Examiner's concern.

Finally, even if each kind of lymphoma had to be tested empirically—and Applicants submit that it is not, because the cells and vaccines provided by the invention contain several different tumor antigens—this would still involve only routine experimentation. A person having ordinary skill in the art only needs to follow the teachings contained in the application to test a particular cancer by using cells expressing MUC1, TF, and glycophorin on their surface in an amount effective for treating the disorder, for example, by eliciting an immune response.

Thus, only routine experimentation is needed to practice the claimed methods over their full scope and the application provides adequate guidance for how this experimentation should proceed.

II. Breadth of the claims

The Examiner states that undue experimentation would be needed to practice the claimed methods of treatment and/or prevention in their current scope. Office Action at 11. The Examiner appears to be arguing that the scope of the claims is overly broad. The Examiner did not identify any subject matter considered to be enabled.

The M.P.E.P. suggests a two-stage inquiry for a rejection based on claim breadth. *See* M.P.E.P. § 2164.08. The first is to determine how broad the claim is with respect to the disclosure. The second inquiry is to determine if one skilled in the art is enabled to make and use the entire scope of the claimed invention without undue experimentation. The M.P.E.P. also instructs that "[i]f a rejection is made based on the view that the enablement is not commensurate in scope with the claim, the examiner should identify the subject matter that is considered to be enabled." *Id.*; *see also* M.P.E.P. § 2164.04.

The breadth of the rejected claims is clear: the cells of the invention (or vaccines derived from them), which express TF, MUC1, and glycophorin, are administered in a therapeutically or prophylatically effective amount to treat or prevent cancers or tumorigenic diseases. As discussed under the last heading, Applicants have demonstrated that lysates of these cells elicit an immune response to TF, MUC1, and glycophorin in an *in vivo* model of the human immune system. Applicants have also demonstrated that these cells display increased sensitivity to lysis by NK cells. Thus,

the skilled artisan would expect that upon administration of the cells, they would be lysed, and the resulting lysate would produce the desired immune response as demonstrated in the Examples. Adapting these teaching to accommodate specific dosages, modes of administration, or different cancers requires only routine skill in the art, and the application provides adequate direction for how this experimentation should proceed.

The Examiner appears to be concerned that the claimed methods may not effectively treat or prevent every cancer. However, "[t]he presence of inoperative embodiments within the scope of a claim does not necessarily render a claim nonenabled." M.P.E.P. § 2164.08(b). The application shows how to practice the claimed methods, so that "[w]ithout undue experimentation or effort or expense the combinations which do not work will readily be discovered and, of course, nobody will use them and the claims do not cover them." *Angstadt*, 180 USPQ at 219. Accordingly, Applicants submit that the claims are enabled over their full scope.

III. Predictability and knowledge in the art

The Examiner cites three references—Jager *et al.*, *J. Clin. Oncol.* 20: 3872-77 (2002)(*Jager*); Czuczman *et al.*, *J. Clin. Oncol.* 17:268-76 (1999)(*Czuczman*); and Carbone *et al.*, *Seminars Cancer Biol.* 14:399-405 (2004)(*Carbone*)—to support the general allegation that treating or preventing lymphoma requires specific therapeutics for each kind of lymphoma. Office Action at 10. The Examiner also cites Goletz *et al.*, *Advances Expt. Med. Biol.*, 535:147-62 (2003)(*Goletz*), to describe a role for TF in liver metastasis and its contemplated use as a tumor marker for immunotherapy. *Id.*

Applicants do not dispute that different therapies are available for treating different lymphomas. Applicants also acknowledge that there may be molecular differences between different types of lymphoma. The claimed methods, however, are designed to *overcome* this potential challenge by inducing an immune response to a *suite* of antigens known to be expressed on tumor cells, namely TF, MUC1, and glycophorin. *See, e.g.*, specification at 4-8; *see also Goletz* (discussing the particular prevalence of TF antigen in a wide variety of tumors); *Ichiyama* (cited in the art-based rejections, below, suggesting that MUC1-transformed K562-derived cells are useful for generating an immune response to tumor cells). Furthermore, evidence suggests that TF is particularly effective as a cancer vaccine when presented in the context of glycophorin. *See* specification at 6.

In addition to recognizing that TF, MUC1, and glycophorin are pan-carcinomic markers, the art also recognizes that immune-based therapies, like Applicants' claimed methods, are effective in cancer treatment. *See, e.g.*, specification at 2; *Goletz* at 156-159 (for TF in particular); *see also Ichiyama* (for MUC1). In fact, *Czuczman,* cited by the Examiner to highlight different lymphoma treatments in the art, demonstrates that immunotherapy can be used to treat lymphoma. There, a combination therapy included administering anti CD-20 *antibodies* that "deplete malignant B cells through complement-dependent cell cytotoxicity, antibody-dependent cell-mediated cytotoxcicity, and apoptotic mechanisms," with beneficial results. *See Czuczman* at 269, left column, second full paragraph and abstract.

The other references cited by the Examiner also fail to support the allegation that Applicants' claims are not enabled. For example, *Jager's* clinical study of treating

mucosa-associated lymphoid tissue lymphoma with chemotherapy showed that 84% of patients in that study achieved complete remission. *See Jager* at abstract. The fact that a different method of treatment is effective does not have a negative bearing on whether the pending claims are enabled—it simply demonstrates one method of treating lymphoma. Moreover, Applicants' methods need not *supplant* existing therapies, such as radiation or other chemotherapies, which may be effective for treating cancers with different etiologies, but can be used *together* with other therapies. *See* specification at 36, lines 8-10.

The Examiner quotes a single passage in *Carbone*, a review article discussing the identification and classification of carcinogens, which merely reiterates the potential problem of treating cancers by a *single-target* approach, because of genetic heterogeneity in cancer. *See Carbone* at 400, left column, bridging first paragraph. Applicants recognized this potential problem and the claimed methods utilize cells that express several pan-carcinomic markers to avoid it.

Goletz supports the enablement of Applicants' claims in several respects. First, it reports that TF is a widely-expressed tumor marker. *See Goletz* at 153, Table 2. Secondly, it describes how TF antigen, in addition to being an excellent *marker* for tumors, may actually play a *functional* role in metastasis in the liver and endothelium. *See Goletz* at 153-55. Finally, it discusses the promising role of TF antigen in cancer immunotherapy, demonstrated, in part, in the present application. For example, *Goletz* describes early success by others in treating advanced breast cancer with enzymatically desialylated glycophorin, which carried high densities of TF. *See Goletz* at 159.

Thus, the art recognizes that the markers on the cells of the invention are widely expressed on tumor cells and that immune-based therapies are effective in cancer treatment. Accordingly, the skilled artisan would expect a reasonable correlation between raising an immune response to these antigens—shown in the working examples—and Applicants claimed methods of treatment.

IV. Direction provided/ working examples

The Examiner states that the specification is silent about *in vivo* administration of a cell line which expresses on the cell surface TF, MUC1 and glycophorin to treat or prevent lymphoma. Office Action at 9. The Examiner alleges that there is insufficient guidance in the application or prior art to support a correlation between administering a cell line of the invention and a therapeutic effect in the treatment or prevention of lymphoma. *Id.* at 11. Thus, the Examiner's rejection appears to be based on the lack of an example conclusively demonstrating *in vivo* efficacy for treating lymphoma with the cells of the invention. Applicants respectfully traverse.

Applicants note again that the presence or absence of a working example is not, by itself, determinative for meeting the enablement requirement of 35 U.S.C. § 112, first paragraph. M.P.E.P. § 2164.02. "The mere fact that something has not previously been done clearly is not, in itself, a sufficient basis for rejecting all applications purporting to disclose how to do it." *Id.*, citing *Gould v. Quigg*, 3 USPQ 2d 1302, 1304 (Fed. Cir. 1987)(internal citation omitted). Requiring Applicants to demonstrate *in vivo* efficacy for treating lymphoma overstates the requirement for patentability that only "a *reasonable* correlation between the activity in question and the asserted utility [is needed]." M.P.E.P. § 2107.03 (I); *see also* M.P.E.P. § 2164.02 ("the [E]xaminer must

also give reasons for a conclusion of lack of correlation for an *in vitro* or *in vivo* animal model example [in an enablement rejection]").

In *Cross v. Iizuka*, 224 USPQ 739, 747 (Fed. Cir. 1985), the Federal Circuit stated that: "in vitro results with respect to the particular pharmacological activity are generally predictive of in vivo test results, i.e., there is a reasonable correlation therebetween. Were this not so, the testing procedures of the pharmaceutical industry would not be as they are." Further clarifying, the Cross Court stated that "a rigorous correlation is *not* necessary...." *Id.*, emphasis added.

The Examiner has not met the initial burden of providing adequate specific technical reasons that would lead the skilled artisan to doubt the correlation between raising *in vitro* and *in vivo* immune responses to several antigens (TF, MUC1, and glycophorin) known to be expressed on tumor cells, and the claimed methods of treatment. Applicants respectfully submit that a skilled artisan would believe that these results reasonably correlate with the claimed methods of treatment because the art recognizes that these antigens are expressed on a wide array of cancers, including lymphoma, and immunotherapy is an accepted approach to treating cancer.

As the Examiner acknowledges, the application discloses, *inter alia*, *in vivo* induction of IgG and IgM antibody responses in NOD/SCID mice reconstituted with human PBMC that were vaccinated with lysates of the cells provided by the invention. *See, e.g.*, specification at 55, lines 24-30; Table 3. This included induction of T helper immune responses and memory immune responses against MUC1, TF, and glycophorin in a model that is nearly a fully-human immune system. These antigens are known to be pan-carcinomic tumor markers. *See* specification at 4; *Goletz* at 152-153,

particularly Table 2; *see also Ichiyama* at 110. Immune-based therapies are known to be useful in cancer treatment. *See, e.g.*, specification at 1-2; *see also Goletz* at 156-159 (describing using TF in a variety of immunotherapies); *Ichiyama* at 110 (immune response to MUC1); *Czuczman* (using CD20 antibodies to treat lymphoma). Accordingly, because the cells of the invention can be used to elicit effective immune responses to antigens shown to be widely expressed on tumors and immune-based therapies are known to be useful in treating cancer, the skilled artisan would expect Applicants' claimed methods to be effective for treating tumors by recruiting a host immune response. Any experimentation that might be required, such as adjusting the dose of cells to accommodate a human subject, is well within the skill of the art. *See, e.g.,* Freireich *et al.*, *Cancer Chemother. Rep.* 50:219-244 (1966), attached.

Thus, no undue experimentation is needed to practice the claimed methods, which are supported by working examples showing how to use the cells of the invention to elicit an *in vivo* immune response to TF, MUC1, and glycophorin. The skilled artisan would expect these results to reasonably correlate with the claimed methods of treatment. Applicants respectfully request withdrawal of the rejection and reconsideration of the claims.

Novelty

Claim 1 was rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Ichiyama, *Kareiigaku Kenkyusho Zasshi* 51(3,4): 93-110 (2000)(*Ichiyama*), as evidenced by Benoist et al., *Immunol. Lett.* 34:45-56 (1992)(*Benoist*), and Karsten et al., *Cancer Res.* 58:2541-49 (1998)(*Karsten*). Specifically, although the Examiner acknowledges that *Ichiyama* does not report that the TF and glycophorin antigens are

on the surface of the K562-derived cells described there, the Examiner references *Benoist* and *Karsten* to supposedly show that glycophorin A and TF, respectively, are "inevitably and inherently" present in K562 cells. Office Action at 12. Applicants respectfully disagree.

Applicants first note that the present application shows that K562 cells, from which the cells in *Ichiyama* are derived, do not express TF antigen. *See*, for example, Figure 1; *see also* specification at 6-7, 10, and 50. In fact, the NM-F9 and NM-D4 cells described in the present application were produced by mutagenizing K562 cells with EMS, and selecting for strong and stable expression of the tumor-specific TF antigen, a property that the parental K562 cells did not possess. *See, e.g.*, Example 2. If this feature did not distinguish NM-F9 and NM-D4 cells from K562 cells, no such selection would be possible. Cotransfection of K562 cells with MUC1 and B7, as described in *Ichiyama*, does not change this fact.

The Examiner cites *Karsten* to allegedly show that TF antigen is present within the immunodominant region of MUC1. *Karsten*, however, only reports that short *synthetic* peptides *derived* from MUC1 and *engineered* to contain TF elicited enhanced binding of some MUC1 antibodies in *in vitro* cell-free ELISAs. *See, e.g., Karsten* at abstract, materials and methods. *Karsten's* report of engineered glycopeptides, however, does not teach (or suggest) that full-length MUC1 transformed into the K562-derived cells of *Ichiyama* contains TF, let alone on the cell's surface, as required by claim 1. In fact, Applicants have provided evidence to show that MUC1 in K562 cells does not express TF antigen. MUC1 from untreated K562 cells was shown to be TF negative and can only exhibit any TF after neuramidase treatment. *See* specification at

52, lines 24-28 and Table 2. In contrast, the cell lines of the invention continuously express high levels of TF. Although Applicants do not wish to be bound by theory, this may be due to a defect in the cell's glycosyltransferases. Specification at 7, lines 1-6. As a result, the cells strongly and stably express this otherwise hidden antigen. Accordingly, *Karsten* does not teach (or suggest) that the MUC1-transformed cells described in *Ichiyama* express the TF antigen and Applicants have provided strong evidence to the contrary.

Thus, *Ichiyama*, as evidenced by *Benoist* and *Karsten*, does not teach all features of claim 1 and can not anticipate it. Accordingly, the rejection should be withdrawn and the claim reconsidered.

Non-obviousness

Claims 1, 5, 6, 11, and 12 stand rejected under 35 U.S.C. § 103(a) as allegedly obvious over *Ichiyama*, in view of *Benoist* and *Karsten*, and further in view of U.S. Patent No. 7,268,120 by Horton, *et al.* Office Action at 13. Specifically, in addition to the allegations discussed under the previous heading, the Examiner further alleges that, with regard to claims 5 and 6, the nucleic acid encoding B7 cotransfected with MUC1 in the K562-derived cells of *Ichiyama* is a costimulatory molecule mediating interactions between T cells and APC. The Examiner further alleges that, with respect to claims 11 and 12, the cells described in *Ichiyama* were used as a pharmaceutical composition, when cocultured with PBMCs. The Examiner acknowledges that the collective disclosure of *Ichiyama*, *Benoist*, and *Karsten* "fails to teach transformation of K562 cells with a vector containing a cytokine, MHC, and others)[sic]." Office Action at 14. The '120 patent was cited to allegedly show that use of *ex vivo* polynucleotide constructs

and selective transfection of malignant cells containing polynucleotides expressing therapeutic of prophylactic molecules was known in the art and that TF and MUC1 were known tumor-associated immunogenic antigens. Allegedly the skilled artisan would be motivated to modify the K562-derived cell line of *Ichiyama* by transfection with a nucleic acid encoding any epitope to enhance the immunogenic response. Applicants respectfully traverse.

As discussed under the previous heading, *Karsten* and *Benoist* do not teach or suggest that the K562-derived cells described in *Ichiyama* have TF, glycophorin, and MUC1 on the cell surface. The present application demonstrates that K562 cells do not express TF antigen. *See, e.g.*, Figure 1. Transforming K562 cells with a plasmid encoding MUC1, as described in *Ichiyama*, does not change this fact, since, unlike the synthetic MUC1-derived glycopeptides engineered to contain TF described in *Karsten*, MUC1 expressed by K562 cells does not contain TF. *See* Table 2 of the instant specification. The '120 patent's report of transforming malignant cells with nucleic acids or merely reciting the terms "TF" and "MUC1" as tumor antigens does not remedy this defect.

Although the '120 patent *recites* the term Thompson-Friedenreich antigen, it incorrectly identifies it in a laundry list of "tumor-associated antigenic and immunogenic *polypeptide*[s]...." U.S. Patent No. 7,268,120 at column 47, lines 62-66. As the Examiner is aware, TF is a carbohydrate-based antigen that is conjugated to certain proteins and is not itself a polypeptide. The '120 patent contains no teaching or suggestion that transformation of a cell with *any* nucleic acid would lead to TF expression. The entirety of the '120 patent never again mentions the TF antigen (or

MUC1) and alone or in combination with *Benoist*, *Karsten*, and *Ichiyama*, offers no teaching, suggestion, or motivation to make a cell line that has TF, glycophorin, and MUC1 on the cell surface, let alone with the necessary reasonable expectation of success. *See* M.P.E.P. § 2143.02.

Moreover, even if the cited references did offer some teaching or suggestion to make the claimed cells—and Applicants submit that they do not—Applicants have shown that the claimed cells exhibit unexpected and beneficial properties, which would rebut a *prima facie* obviousness rejection. *See, e.g.*, M.P.E.P. § 2145. For example, lysates of the cells provided by the invention have been shown to advantageously induce immune reactions to, *inter alia*, TF, MUC1, and glycophorin—both *in vitro* and *in vivo*. *See, e.g.*, Example 4 and Table 3. Surprisingly, this immune reaction is mediated by IgG, in addition to IgM. Induction of an IgG response indicates a switch of antibody class associated with a T helper cell immune response as well as induction of memory immune responses against these antigens and is highly desirable. *See*, *e.g.*, specification at 24, lines 4-10 and Example 4B-1 at 55.

In sum, the collective disclosure of *Ichiyama*, *Benoist*, *Karsten*, and the '120 patent do not teach or suggest every element of Applicants' claims, let alone the unexpected, but desirable, IgG response that results from using the claimed cells as described in the application. Accordingly, these references do not render the pending claims obvious. Applicants respectfully request withdrawal of the rejection and reconsideration of the claims.

Applicants do not believe any fees are required to enter this amendment. However, Commissioner is authorized to charge any required fees to deposit account number 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: April 27, 2009

By: _____
Laurence A. Shumway, Ph.D.
Reg. No. 61,169
617.452.1689

Attachment:

Deposit Declaration by Assignee Glycotope GmbH

Freireich *et al.*, *Cancer Chemother. Rep.* 50:219-244 (1966)

Attachment 1

Attorney Docket No. 10913.0003-00000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Steffen Goletz et al.	)	Group Art Unit: 1633
	)	
Application No.: 10/568,098	)	Examiner: Leavitt, Maria Gomez
	)	
Filed: June 20, 2006	)	Confirmation No.: 8158
	)	
For: TUMOR CELL LINES AND USES THEREOF	)	
	)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DEPOSIT DECLARATION

I, Dr. Hans Bastert, do hereby declare:

1. Glycotope GmbH is the assignee of the above-identified patent application as evidenced by an assignment recorded on June 15, 2006, at Reel 17789, Frame 0210.

2. On information and belief, cell lines "NM-F9" and "NM-D4" were deposited under the provisions of the Budapest Treaty at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (D.S.M.Z.) at Inhoffenstr. 7 B, 38124 Braunschweig, Germany, on August 14, 2003. Cell line NM-F9 was assigned deposit accession number DSM ACC2606. Cell line NM-D4 was assigned deposit accession number DSM ACC2605.

3. Copies of the D.S.M.Z. deposit receipt, viability statement, and taxonomic identification form for each of cell lines NM-F9 and NM-D4 are attached as Appendix A, dated 6.6.03 and Appendix B, dated 6.6.03, respectively.

4. On information and belief, the D.S.M.Z. has acquired the status of International Depository Authority within the meaning of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of the Patent Procedure.

5. On information and belief, the D.S.M.Z. is a depository affording permanence to the deposit and ready accessibility thereto by the public if a patent is granted.

6. On information and belief, the material has been deposited under conditions that ensure that access to the material will be available during the pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 C.F.R. 1.14 and 35 U.S.C. § 122.

7. On information and belief, the deposited material will be stored with all care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the deposited microorganism, and in any case at least thirty (30) years after the date of a deposit or for the enforceable life of the patent, whichever is longer.

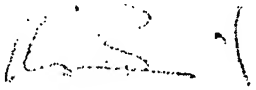
8. On information and belief, all restrictions on the availability to the public of the deposited cultures will be irrevocably removed no later than the granting of a patent from the above-identified application.

9. I acknowledge Glycotope GmbH's duty to replace the deposited culture should the depository be unable to furnish a sample when requested due to the condition of the deposit during the period that extends thirty (30) years from the date of the deposit, or the period of the enforceable life of the patent, or the period of five years after the last public request for the deposit, whichever period is longest.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title of 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

11. The undersigned is authorized to sign on behalf of assignee, Glycotope GmbH.

Signed this 9th day of April, 2009.

Signed: 

Title: CEO

Attachment 2

cancer chemotherapy reports

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QUANTITATIVE COMPARISON OF TOXICITY OF ANTICANCER AGENTS IN MOUSE, RAT, HAMSTER, DOG, MONKEY, AND MAN[1,2]

Emil J Freireich,[3] Edmund A. Gehan,[4] David P. Rall,[5] Leon H. Schmidt,[6] and Howard E. Skipper[7]

SUMMARY

Toxicity data from small animals (mouse, rat, and hamster), large animals (dog and monkey), and humans were gathered, placed on a reasonably similar basis, and compared quantitatively. Each animal species and all species combined were used to predict the toxic doses in man (based on mg/m² of surface area). Two models were assumed for the relationship between the maximum tolerated dose (MTD) in man and the approximate LD10 in each animal system:

$$(\text{dose in man}) = (\text{dose in animal system } i) \quad (1)$$

and

$$(\text{dose in man}) = A_i \times (\text{dose in animal system } i), \ (i = 1, \ldots, 6) \quad (2)$$

where A_i is the fraction of the dose in animals used to predict the dose in humans (assumed different for each animal system, ie, $i = 1, \ldots, 6$). It was found that when animal systems other than the rat were used the very simple model (1) was remarkably good for predicting the MTD in humans, though model (2) leads to slightly better predictions. Based on model (2), the animal systems are ranked in order of predictive ability: rhesus monkey, Swiss mouse, rat, BDF_1 mouse, dog, and hamster. The best estimate of the MTD in man is made by weighting the estimates from the various animal species. Dose on an mg/m² basis is approximately related to dose on an mg/kg basis by the formula

$$(\text{dose in mg/m}^2) = (km)_i \times (\text{dose in mg/kg}), \quad (i = 1, \ldots, 7)$$

where $(km)_i$ is the appropriate factor for converting doses from mg/kg to mg/m² surface area for each species. When the $(km)_i$ factors are known, equally good predictions of MTD in man can be made by either dose unit. On an mg/m² basis, the MTD in man is about the same as that in each animal species. On an mg/kg basis, the MTD in man is about $\frac{1}{12}$ the LD10 in mice, $\frac{1}{9}$ the LD10 in hamsters, $\frac{1}{7}$ the LD10 in rats, $\frac{1}{3}$ the MTD in rhesus monkeys, and $\frac{1}{2}$ the MTD in dogs. In each case the ratio is the (km) factor in the animal system to that in man. Hence relationships among the various animal species and man are somewhat simpler and more direct on an mg/m² basis. These results support the conclusion that the experimental test systems used to evaluate the toxicities of potential anticancer drugs correlate remarkably closely with the results in man.

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[1] Received Dec 29, 1965; revised Jan 17, 1966.

[2] Study done under the auspices of the Acute Leukemia Task Force of the National Cancer Institute by the Subhuman Subcommittee.

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The biologic aspect of a drug development program to discover compounds effective against any clinical disease is generally an exercise in comparative pharamacology. In the typical program, compounds are screened in small animals against some easily produced and reproduced pathologic condition. A close relationship must exist between the screening system and the ultimate clinical condition for the program to have the potential for success. Thus examination of this relationship is highly important. In cancer chemotherapy the similarities and differences have often been considered among transplantable tumors, virus-induced tumors, carcinogen-induced tumors, and spontaneous tumors in animals, and between animal tumors and the various cancers and leukemias in man. However the similarities and differences between mice, rats, hamsters, dogs, monkeys, and man have been considered less often in terms of quantitative and qualitative aspects of the toxic effects of drugs. The consistency of the action of therapeutic agents among various mammalian species is a keystone of most drug development programs, yet only rarely has this been studied in a quantitative manner.

Classically comparative pharmacology and physiology have been concerned with differences which permit analytic studies of specific biologic systems, and these studies have yielded valuable information. But it is equally important to consider the much more frequent similarities; we have tried to do this in the present analysis.

Of all the toxicologic end points, lethal toxicity is the easiest to measure with reasonable precision. Therefore we considered the lethal dose of certain cancer chemotherapeutic agents in various laboratory animals. For man the end point was the maximum tolerated dose (MTD). Hopefully two benefits might accrue from this evaluation: (1) If there is reasonable consistency in the reactions of various mammalian species, the toxicologic component of cancer chemotherapy screening will be shown to have a rational basis. (2) If such consistency is found, the problems of introducing highly toxic therapeutic agents into man might be approached more confidently. If major inconsistencies are discovered frequently, this would highlight the deficiencies in present screening systems and raise serious questions about the utility of these schemes for safe introduction of new drugs into man.

No attempt was made to relate therapeutic doses in the various mammalian species. In the future this correlation should be attempted since the therapeutic target in the host is not the same as the toxicity target. However if an agent has therapeutic properties in an experimental system, it is well to know the dose level for patients. Since there is some justification for using MTD's in cancer therapy, these dose levels were studied.

The plan of this retrospective study was to examine considerable toxicologic data obtained in (*a*) small animals, used in primary screening and quantitative secondary drug evaluation; (*b*) larger animals, dogs and monkeys, for the quantitative and qualitative aspects of toxicity at sublethal and lethal levels; and (*c*) man, the target species. The goal was to determine what relationship exists, if any, between certain commonly used toxicologic end points in the various animal species and man for a number of anticancer agents.

Nothing in this report is intended to suggest or imply that short cuts are allowable in preclinical or clinical toxicologic studies. Dose-limiting and serious toxic effects in man are not always apparent from even the most carefully done toxicologic investigations in animals (1). *It is emphasized and should be clearly understood that it is dangerous to attempt to extrapolate directly from animal toxicity data to maximum tolerated doses in man!* New drugs can be introduced safely into clinical trial only through careful toxicologic and pharmacologic study in animals and then very cautious study in man, starting with much lower dosages than those which appear to be tolerated by the animals.

APPROACHES AND ASSUMPTIONS IN THIS STUDY

The published and unpublished data which form the basis for this analysis were obtained by numerous investigators using different protocols and end points. We used consistent and reasonable general assumptions so that the data were comparable. The biologic end points, protocols, assumptions, and corrections necesssary to make the results more comparable are described briefly.

Toxicologic End Points (See Appendix I).

Mouse, rat, or hamster: Lethality—the dose which when administered by a certain route and schedule killed a selected percentage (10%, ie, the LD10) during a specified observation period; 50 to more than 100 animals were used in a typical determination.

Dog or monkey: (*a*) MTD; typically 2–4 animals were used at each dose level, spaced by 2-fold increments. In all instances individual doses which killed 0 and 100% were used. The highest dose killing 0% was considered the MTD. (*b*) Dose-related, hematopoietic effects; localized hemorrhages of the gastrointestinal tract; generalized hemorrhagic lesions (abdominal and thoracic viscera); stimulation of the central nervous system (CNS); others.

Man: (*a*) MTD for a fixed schedule (dose causing mild to moderate sublethal toxic effects in a significant percent of patients); (*b*) MTD for a variable schedule, calculated from the daily dose and median period to toxic effects requiring cessation of drug; the judgment of many clinical investigators was necessarily accepted in making this estimation.

Because of the nature of the available data, the toxicologic end points in the various animal species were related to the MTD in man. Although it was necessary to assume that the dosages resulted in the *same* percentage of toxicity in each species, the results do not depend, in a major way, on this assumption. For the drugs in this study, the dose-toxicity curves were relatively steep so that if the true percentage of toxicity for a given dosage was, say, between 5% and 15%, the actual dosage used would not differ very much from the dosage that should have been used.

It was necessary to use toxicologic data obtained by various routes of drug administration, ie, intraperitoneal (ip) for small animals, oral for small animals and man, and intravenous (iv) for large animals and man. In mice and rats the LD10's obtained by the ip and iv routes are usually comparable.

Another variable for which some reasonable correction must be made is the dosage schedule including the total dose. We assumed that the toxicity of anticancer agents is cumulative. Griswold et al. (3) reported that when the LD10's in BDF_1 mice of 70 agents, including the major classes of anticancer agents, were compared for two schedules, qd 1–7 days and qd 1–11 days,[8] the mean ratio (qd 1–7 days/qd 1–11 days) was 1.56. This is very close to that which might be expected from direct cumulative drug toxicity (11 days/7 days = 1.57).

Pinkel (2) and other investigators pointed out that the usual doses of certain drugs in various animal species and man were comparable when the dose was measured on the basis of mg/m² of surface area. Consequently most of the results are presented in mg/m². However since mg/kg is a commonly used unit of drug dosage, some results are also presented in this unit. Only a simple transformation is required to change mg/kg to mg/m²; therefore the relationships developed are equivalent whichever unit is used. The quantitative relationships were simpler when expressed in mg/m².

A conversion factor (km) was used to transform mg/kg to mg/m² by the equation mg/kg × (km) = mg/m²; (km) factors for animals, given their weight, are presented in table 1 (Appendix II), and table 2 (Appendix II) presents a way of transforming doses in mg/kg to mg/m² for man, given height and body weight. Chart 1 (Appendix II) is a diagram for determining surface area in man, given height and weight.

Calculations based on units of body surface area have no intrinsic merit per se. Very likely some other basis such as surface area of the site of action of the drug, lean body mass, or some fractional power of body weight, possibly related to length or some organ-membrane surface area, would be as appropriate or more appropriate. However the body surface area has been used to relate many physiologic parameters among species and means of transforming the data are readily available. Further, in our clinical studies we routinely use body surface area to adjust drug dose for patients of different size and weight.

RESULTS

The first step in analyzing the data was to correct the daily dosage schedules for man and for animals, when necessary, to a uniform schedule of qd 1–5 days. Thus if an LD10 for mice, or MTD for man, was obtained by a schedule of qd 1–10 days, we calculated that the LD10 (or MTD) for a schedule of qd 1–5 days was twice that value. The next step was to convert doses (LD10's or MTD's) from mg/kg to mg/m². This was accomplished by the approximate formula

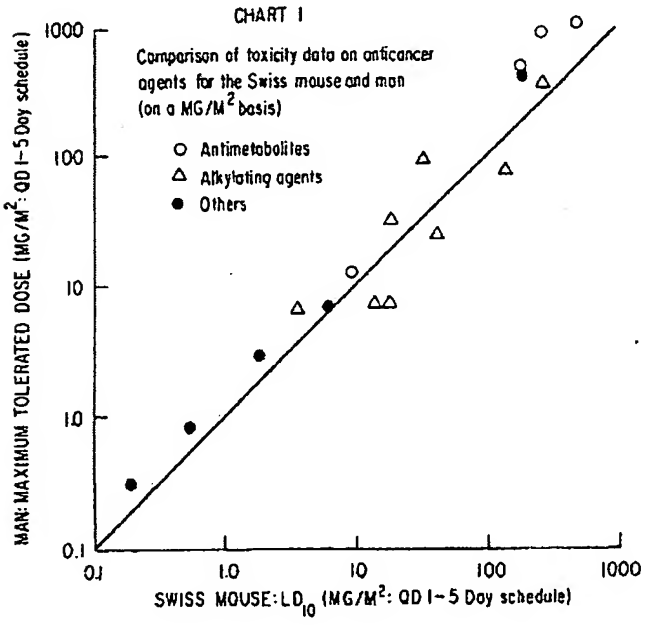
$$(\text{mg/m}^2) = (km)_i \times (\text{mg/kg}), \quad (i = 1, \ldots, 7)$$

where the $(km)_i$ factor differs according to the species and also according to body weight within each species. In the analysis an average $(km)_i$ factor was used, assuming that individuals in each species were of average height-to-body-weight ratios. The $(km)_i$ factors were derived from standard relationships between weight and surface area as given in Spector (40) and Sendroy and Cecchini (39). Details and other information on relating drug doses in mg/kg to doses in mg/m² are given in Appendix II.

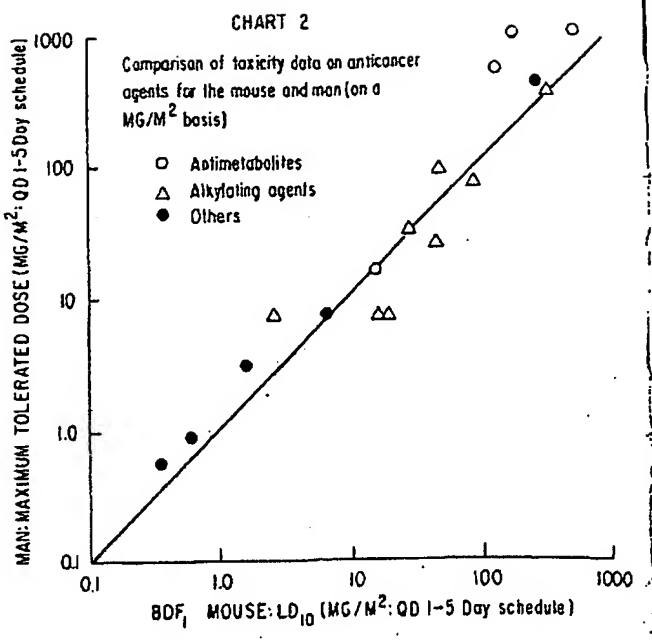
[8] qd = drug given once daily for as many days as indicated.

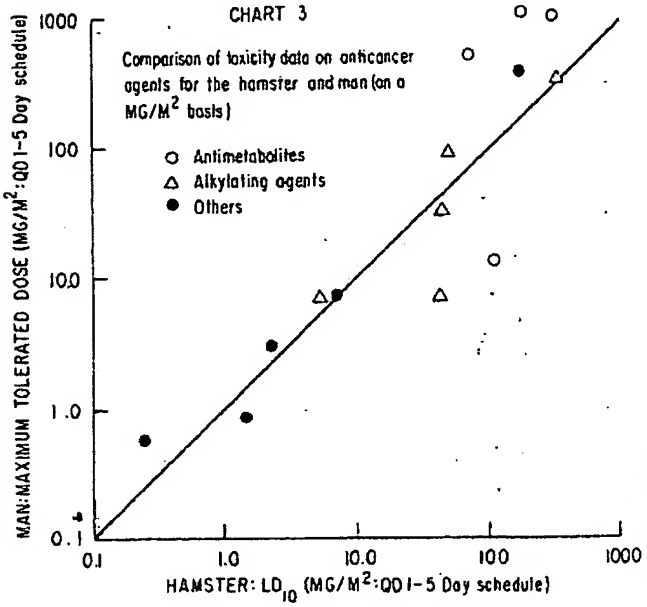
The basic data used in this study are given in table 1. Doses of 18 drugs[3] are presented in mg/kg and mg/m² for the 6 species, along with source information and other pertinent data. An average dose (LD10 or MTD) of each drug was calculated from the multiple studies, if done, on each species. The average doses for the 6 animal systems and man are given in mg/kg in table 2, and in mg/m² in table 3. Charts 1–6 indicate the closeness of the relationship between the logarithm of the LD10, or MTD, in the various animal systems and in man when the dose is measured in mg/m². Chart 7 indicates the close relationship between 12 times the LD10 in the BDF_1 mouse and the MTD in man when the dose is measured in mg/kg. The ratio of the (km) factors for an average man and a mouse is 37/3 = 12.3. It will be shown later that relationships between systems on an mg/kg basis are the same as those on an mg/m² basis if the ratio of (km) factors is considered.

To examine further the relationship of dosage, in mg/m², between the animal systems and man, consider the following: For each animal system and man, there is a dose-toxicity curve. The basic data for each drug consist of estimates of a single point, the approximate LD10, on the dose-toxicity curves for man and the 6



[3] *Chemical Abstracts'* nomenclature and NSC numbers for the agents are given on page 243.





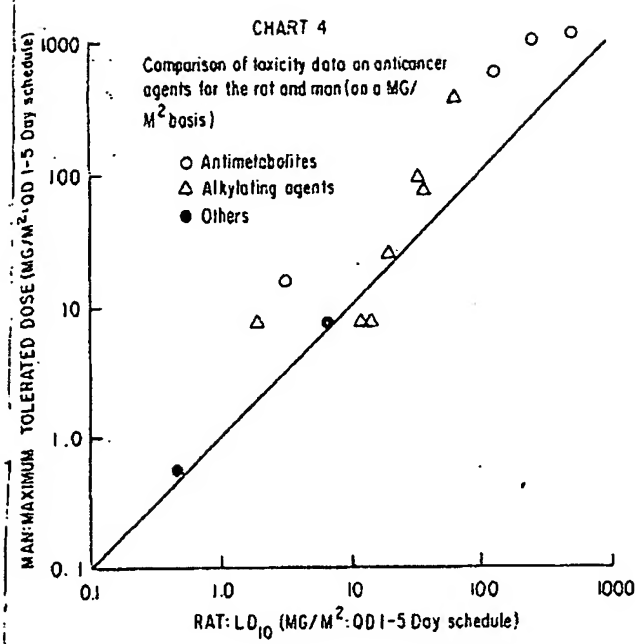
animal systems. We wish to describe the relationship between the dose-toxicity curve for man and that for each of the animal systems. Two models are considered:

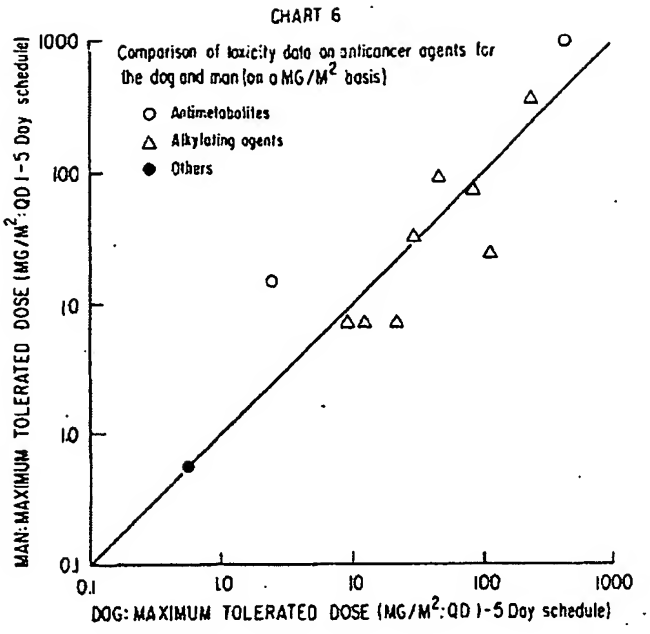
$$\text{(dose in man)} = \text{(dose in animal system } i) \quad (i = 1, \ldots, 6) \qquad (1)$$

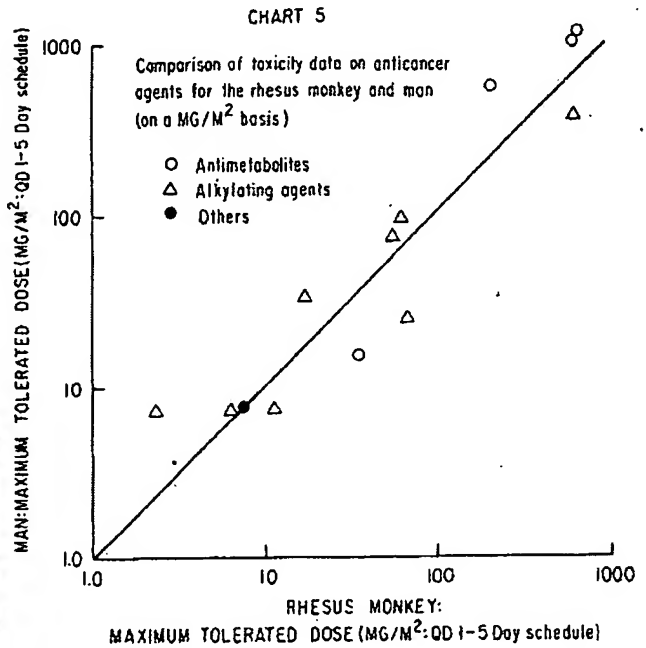
and

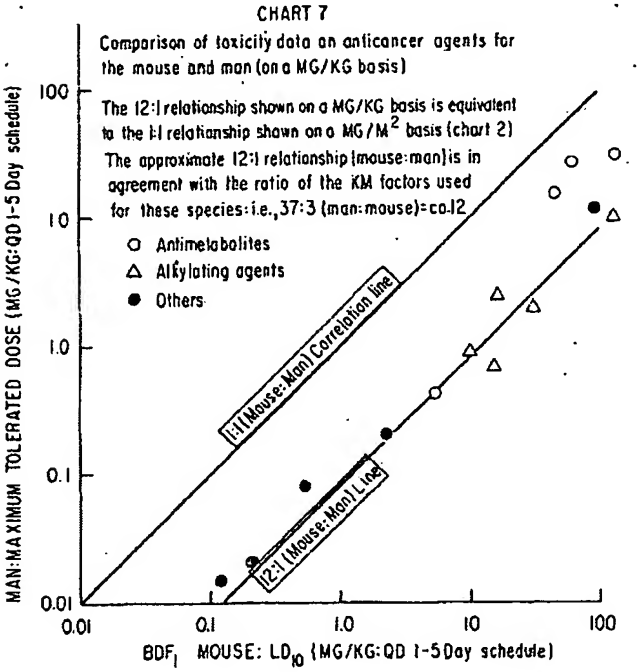
$$\text{(dose in man)} = A_i \times \text{(dose in animal system } i), \quad (i = 1, \ldots, 6). \qquad (2)$$

Model (1) is a special case of model (2) since they are the same when $A_i = 1$. Model









(1) assumes that the dose in each animal system gives a direct prediction of the dose in man. Model (2) assumes that the dose in man is a fraction (A_i) of the dose in the animal system and the fraction remains constant for the sample of drugs.

A third model was considered:

$$(\text{dose in man}) = A_i \times (\text{dose in animal system } i)^{B_i}, \quad (i = 1, \ldots, 6)$$

where B_i is the power to which the dose is raised, assumed to be 1 in models (1) and (2). This model is a natural generalization of (2). However, since the estimates of B_i were near 1 for all animal systems, in fact within 1 standard error (SE) limit, there is no advantage to using a more general model than (2).

By these models, we wish to predict the dose in man from the dose in each animal system when both determinations are subject to sampling variation (and other assumptions as men-

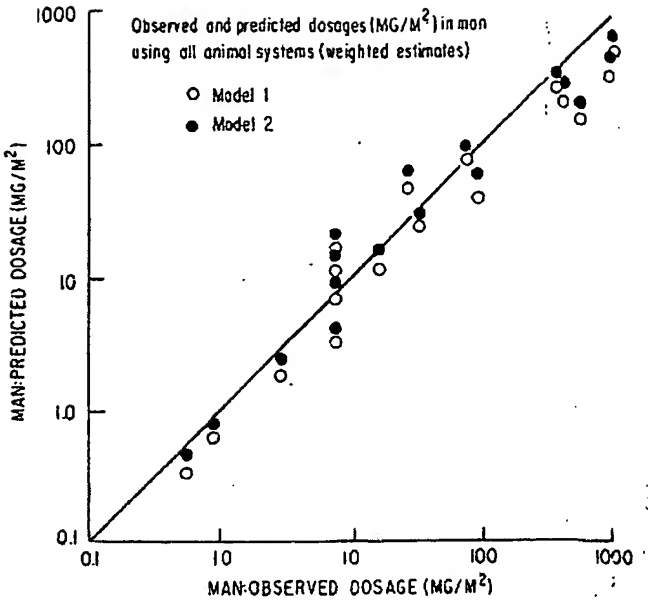
tioned) in the sample of drugs. The statistical considerations in fitting these models are given in Appendix III.

Model (1) is the simplest possible model; no parameters need to be estimated. Thus the doses in table 3 for each animal system are the predicted values of the dose in man and charts 1–6 indicate that these predictions are reasonably good. The standard deviations, on a log scale, of a predicted value of log (dose in man) were calculated for each animal system. The systems are ranked in order of predictive ability in the top half of table 4: monkey, Swiss mice, BDF_1 mouse, dog, rat, and hamster. A predicted value of the dose in man has been calculated by weighting the estimates from each animal system (see Appendix III) and the results are given in the last column of table 3. The standard deviation of a predicted value of log (dose in man) is 0.299, with multipliers of 0.50 and 2.0 for lower and upper standard deviation limits respectively. Thus the weighted estimate based on all systems is better than the estimate from any single system.

Assuming model (2), the estimates of A_i and $A_i \pm 2$ SE are given in the bottom half of table 4. Note that the approximate 95% confidence limits for the multiplying factor, A_i, include 1 for all animals systems except the rat. Thus for the other animal systems it is reasonable to accept the very simple model (1) as providing an adequate prediction of the dose in man. However when all systems are combined to obtain an overall estimate of A_i (see Appendix III), the approximate 95% confidence limits do not include 1. Also, note from the bottom half of table 4 that the standard deviation of a predicted value of log (dose in man) is 0.275, almost a 10% reduction from that of model (1). Therefore model (2) is preferred for fitting these data; however for future studies in which more precise estimates of LD10 are available, it may be that model (1) will be adequate.

Using model (2), we can rank the animal systems in order of their predictive ability by considering the deviations of observed from predicted values of dose in man. These standard deviations are given in table 4. Thus the order is monkey, Swiss mouse, rat, BDF_1 mouse, dog, and hamster. The best predictions with model (2) are obtained by weighting the estimates of the dose in man from all 6 animal systems (the method is explained in Appendix III). The predictions for the drugs in this study are given

CHART 8



in table 5 and the weighted estimates based on all animal systems combined are plotted in chart 8. The best estimates of dose in man, as indicated by the standard deviations in table 4, are given by weighting the individual estimates from each animal system.

Another model was considered in which the dose in man (mg/m²) was related to doses in the animal species in a single equation:

$$\begin{aligned}\log(\text{dose in man}) = {} & 0.284 + 0.847 \log(\text{dose in Swiss mouse}) \\ & - 1.064 \log(\text{dose in } BDF_1 \text{ mouse}) \\ & + 0.539 \log(\text{dose in rat}) \\ & + 0.801 \log(\text{dose in monkey}) \\ & - 0.175 \log(\text{dose in dog}).\end{aligned}$$

This predicting equation leads to a slight improvement in the prediction of the dose in man; the deviations of observed from predicted dosages were less (standard deviation of 0.249 on log scale compared to 0.275 by using weighted, combined estimates). However a prediction of dosage in man cannot be made unless estimates of LD10 are available from all the animal systems mentioned; also the model does not provide any real insight into the relationship between the dose-toxicity curve in each animal system and that in man.

From considering charts 1–6, this question arose: Do the differences between the dose-

toxicity curves for man and for each animal system differ depending on whether an antimetabolite or an alkylating agent was given? Usually the animal species, except the rat and monkey, underpredict the doses of antimetabolites and overpredict the doses of alkylating agents for man. By a statistical test (t test), there was some suggestion ($P<0.10$) that in Swiss mice and BDF_1 mice the predictions of dosage in man were lower for antimetabolites than for alkylating agents. There was no evidence of a difference in the other species. Only 4 antimetabolites and 8 alkylating agents were tested in all animal species. Consequently further study is needed to determine whether the difference between dose-toxicity curves really depends on the type of agent.

There is some value in comparing the relationships found on an mg/m² basis with what would have been found on an mg/kg basis. Some indication of this has already been given in chart 7 which shows that there is a close relationship between 12 times the LD10 in the BDF_1 mouse and the MTD in man. Since the relationship between mg/kg and mg/m² used is

$$(\text{mg/m}^2) = (km)_i \times (\text{mg/kg}), \quad (i = 1, \ldots, 7),$$

models (1) and (2) become, in terms of mg/kg,

$$(\text{dose in man}) = \frac{(km)_a}{(km)_m} \times (\text{dose in animal system}) \qquad (1)$$

and

$$(\text{dose in man}) = \frac{(km)_a}{(km)_m} A_i \times (\text{dose in animal system}) \qquad (2)$$

where $(km)_a$ and $(km)_m$ refer to the (km) factor in the particular animal system and man respectively, and A_i is exactly the same as stated before. Hence it should be clear that dose in man can be predicted equally well either on an mg/kg basis or on an mg/m² basis. Thus by using the km factors and model (1), the dose in man (mg/kg) is approximately $\frac{1}{12}$ the dose in mice, $\frac{1}{9}$ the dose in hamsters, $\frac{1}{7}$ the dose in rats, $\frac{1}{3}$ the dose in rhesus monkeys, and $\frac{1}{2}$ the dose in dogs.

DISCUSSION

Originality is not claimed or implied for this analysis. We have confirmed and extended the general observations and conclusions of Pinkel (2) who confirmed and extended specific aspects of the basic observation of Rubner (36), made 80 years ago, and many other investigators later.

The availability of much more extensive toxicity data from the Cancer Chemotherapy National Service Center program, from certain other published sources, and from our own laboratories seemed to make this present analysis timely. Also we believe it is important to use more definitive biologic end points of toxicity. This analysis and study of data on toxicity to animals and humans of several types of anticancer agents (tables 1, 3, and 5) lead us to conclude that the toxic dose of an agent is similar among species when the dose is measured on the basis of surface area. The skin surface area was used here though it is unlikely that the skin is the target area of action of any particular drug. More likely the skin surface is more or less proportional to the true target surface.

To the extent that mammalian species are broadly similar and have corresponding organs and tissues, it is true that any surface area will increase approximately with the two-thirds power of weight (38). Thus the two-thirds power of body weight would have been a convenient unit of surface area to use and the results of the analysis would have been almost the same (see Appendix II).

Pinkel (2) suggested that "cancer chemotherapists consider the applicability of body surface area as a criterion of drug dosages in their laboratory and clinical studies." We suggest that a unit proportional to body surface area is sufficient and an appropriate unit is $(\text{weight})^{\frac{2}{3}}$.

We have been concerned only with comparisons among species, not within species, and with adult animals, not immature and adult animals. Also we have been concerned solely with anticancer drugs.

Some of the toxicologic data tabulated may disagree with unpublished and published observations of some experimentalists and clinicians. The Acute Leukemia Task Force of the National Cancer Institute wishes to correct, update, and extend this analysis at some future time. Those interested in seeing such correlation efforts extended can help by providing ad-

ditional data, both clinical and experimental, in a form similar to that in table 1.

The present study has emphasized the quantitative aspects of toxicity of anticancer drugs to animals and man. Regarding the prediction of the qualitative effects of anticancer drugs in man from laboratory animal studies, Owens (1) suggested:

Predictive value	*Preclinical toxicity studies*
Good	Bone marrow, gastrointestinal tract, liver, kidney
Questionable	Nervous system, including peripheral neuropathy, extraocular palsies, and CNS toxicity
None	Skin and appendages, including skin rashes, dermatitis, and alopecia

Of the 18 agents in this study, 17 produced limiting toxicity to the bone marrow (marrow depression: MD) and to the gastrointestinal (GI) tract. If the mg/m² doses in man that are predicted by using the weighted combined estimate are compared to the observed doses, then the largest ratio of predicted dose/observed dose is 3, for thioTEPA. Consequently it would be reasonable to study preclinical toxic effects in the mouse, rat, dog, monkey, and hamster, to estimate the MTD (mg/m²) in man, and to start clinical cancer chemotherapy trials at about one-third the predicted dose. This would have been a safe procedure for all 18 drugs mentioned. Owens (1) suggested that it might be reasonable "to begin a human trial at one-tenth of the maximum tolerated dose in the most susceptible animal" (on an mg/kg basis). Since the most susceptible animal will ordinarily be the dog or rhesus monkey, Owens' rule of thumb on an mg/m² basis becomes: begin trial in man at about one-third the dose for monkeys or one-fifth the dose for dogs. Thus there is reasonable agreement between the two recommendations. However if the animal data are not placed on the mg/m² basis before using Owens' rule of thumb, any additional knowledge which the small animals (mouse and rat) might contribute will be overlooked. Remember also that the toxicity values (LD10's) for such small animals are often more reliable statistically because more animals are generally used.

The ratios of animal/human toxicity (mg/m² basis) for the mouse, hamster, dog, and monkey are remarkably close to unity. Thus each species generally predicts for man. That this is true for the mouse is particularly pertinent to cancer chemotherapy. Extensive drug development programs which use mouse tumors seem to be on firmer ground than we had previously thought. In general the rat is more susceptible to these agents than the other species. The hamster is unusually resistant to amethopterin and sensitive to the fluorinated pyrimidines. The dog and monkey, long known to be reasonably good predictors of toxicity to humans, have shown up well in this analysis.

We are *not* suggesting that it is wise to take mouse or rat LD10's, convert the doses to mg/m², and then start clinical trials at one-third this level (in mg/m² for man). The additional safety provided by toxicity data from multiple species is well established, *as is the value of specific qualitative knowledge on dose-related sublethal toxicity and its reversibility.*

Finally it is suggested that the quantitative relationships between toxicity to animals and to humans are simpler when compared on an mg/m² basis than on an mg/kg basis. Broader use of a surface area unit, either mg/m² or (weight)$^{2/3}$, by experimental and clinical cancer chemotherapists, as well as biochemists and pharmacologists concerned with mechanism studies, might prove helpful in many types of experimental planning and data analysis.

Table 1

Comparison of Approximate Maximum Human Dosages of Certain Anticancer Agents with LD_{10}'s for the Mouse, Rat, and Hamster and Approximate Maximum Nonlethal Doses for the Dog and Monkey

Agent	Species[a]	No. of Patients or Animals	Drug Administration: Route	Drug Administration: Schedule (days)	Period of Obs. of Animals for Toxicity in Days; or Median Days to Max. Toxicity in Man	Toxicologic End Point for Species Indicated	Brief Toxicity "Rating" in Man: and Intensity of Major Reactions in Large Animals Rating[b]: 0	Mild	Mod.	Severe	Limiting Toxicologic Symptoms or Reactions	Daily Dose (mg/kg)	Daily Dose "Corrected" to qd 1-5 day Schedule (mg/kg)	"Corrected" Dosage Level (qd 1-5 days) Converted to Surface Area Basis[c]: km Factor	mg/m²	Ratio (mg/m²)/(Animal)/Man	Reference
1. Amethopterin	Man	63	I. V. or I. M.	qd 1-5	11	MTD					MD; GI	0.42	0.42	37.0	16.0		Herta, Lewis, and Lipsett (4)
		22	I. V.	qd 1-5	9	MTD	0	7	14	1	MD; GI	0.41	0.41	37.0	15.0[d]		"Bike" program, NCI (5)
		Very large no.	Oral	qd 1-20		Usual dose					MD; GI	0.10	0.40	37.0	14.8		Karnofsky (6)
	Swiss mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						3.5	3.5	3.0	10.5	0.70	Schmidt (7)
	BDF_1 mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						4.7	4.7	3.0	14.1	0.94	Schmidt (7)
	Swiss mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						2.0	2.8	3.0	8.4	0.56	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						4.6	6.4	3.0	19.2	1.28	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-11	1-21	LD_{10}						2.1	4.6	3.0	13.8	0.92	Griswold (3)
	Hamster	50-100	I. P.	qd 1-7	1-14	LD_{10}						18.0	25.2	4.1	103.0	6.9	Griswold (8)
	H. Rat[e]	50-100	I. P.	qd 1-5	1-21	LD_{10}						0.64	0.64	5.2	3.3	0.32	Schmidt (7)
	F. Rat[e]	50-100	I. P.	qd 1-5	1-21	LD_{10}						0.65	0.65	5.2	3.4	0.23	Schmidt (7)
	S. D. Rat (250 g)	50-100	I. P.	qd 1-15	1-36	LD_{10}						0.13	0.40	7.0	2.8	0.19	Rall (9)
	S. D. Rat (88 g)	50-100	I. P.	qd 1-15	1-36	LD_{10}						0.21	0.64	4.6	2.9	0.19	Rall (9)
	Dog	18	I. V.	qd 1-15	1-36	MTD		MD		GI		0.04	0.12	19.8	2.4	0.16	Rall (9)
	Monkey	7	I. V.	qd 1-15	1-36	MTD			GI; MD			1.0	3.0	11.5	35.0	2.3	Rall (9)
2. 6-Mercaptopurine	Man	18	I. V.	qd 1-5	11	MTD	1	8	8	1	MD	27.0	27.0	37.0	1000[d] (1000-2000)		NCI (5)
		16	I. V.	qd 1-5	10	MTD	1	9	6	0	MD	12.0	12.0	37.0	450 (300-750)		NCI (5)
		Large no.	Oral	qd 1-20		Usual dose					MD	2.5	10.0	37.0	370.0		Karnofsky (6)
	Swiss mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						ca 90.0	ca 90.0	3.0	ca 270.0	ca 0.27	Schmidt (7)
	BDF_1 mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						62.0	62.0	3.0	186.0	0.18	Schmidt (7)
	Swiss mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						58.0	81.0	3.0	243.0	0.24	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						44.0	62.0	3.0	186.0	0.19	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-11	1-21	LD_{10}						28.0	62.0	3.0	186.0	0.19	Griswold (3)
	Hamster	50-100	I. P.	qd 1-7	1-14	LD_{10}						56.0	78.0	4.1	320.0	0.32	Griswold (8)
	H. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						54.0	54.0	5.2	281.0	0.28	Schmidt (7)
	F. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						ca 48.0	ca 48.0	5.2	ca 250.0	ca 0.25	Schmidt (7)
	Dog	10	I. V.	qd 1-4	1-15+	MTD		MD; GI				17.5	21.9	19.8	434.0	0.43	Philips et al. (10)
	Monkey	4	I. V.	qd 1-7	1-60	MTD		MD; GI				40.0	56.0	11.5	644.0	0.64	Rall (9)
3. 5-Fluorouracil	Man	1300	I. V.	qd 1-5[f]	7-21	MTD	0%	97.5%		2.5%	GI; MD	15.0	15.0	37.0	555.0[d]		Ansfield (11)
		200	I. V.	qd 1-5[g]	14-28	< MTD	30%	70.0%		0%	GI; MD	12.0	12.0	37.0	444.0		Ansfield (11)
		223	I. V.	qd 1-5[g]	21	MTD	0%	41%	56%	3%	GI; MD	15.0	15.0	37.0	555.0		Moertel et al. (12)
		Large no.	I. V.	qd 1-5		Usual dose					GI; MD	15.0	15.0	37.0	555.0		Karnofsky (6)
	Swiss mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						31.0	31.0	3.0	93.0	0.17	Schmidt (7)
	BDF_1 mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						ca 30.0	ca 30.0	3.0	ca 80.0	ca 0.16	Schmidt (7)
	Swiss mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						36.0	53.0	3.0	159.0	0.29	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						33.0	46.0	3.0	138.0	0.25	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-11	1-21	LD_{10}						27.0	59.0	3.0	177.0	0.32	Griswold (3)
	Hamster	50-100	I. P.	qd 1-7	1-14	LD_{10}						12.0	17.0	4.1	70.0	0.13	Griswold (8)
	H. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						25.0	25.0	5.2	130.0	0.23	Schmidt (7)
	F. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						25.0	25.0	5.2	130.0	0.23	Schmidt (7)
	Dog	8	I. V.	qd 1-10	1-40	MTD						5.0	10.0	19.0	190.0	0.39	Philips (37)
	Monkey	6	I. V.	qd 1-6	1-60	MTD		MD; GI				15.0	18.0	11.5	207.0	0.37	Rall (9)
4. 5-FUDR	Man	200	I. V.	qd 1-5[f]	14-28	< MTD	30%	78%		2%	GI; MD	30.0	30.0	37.0	1110.0[d]		Ansfield (11)
		51	I. V.	qd 1-5[g]	21	MTD	0%	33%	63%	4%	GI; MD	40.0	40.0	37.0	1480.0		Moertel et al. (12)
		Large no.	I. V.	qd 1-5		Usual dose					GI; MD	30.0	30.0	37.0	1110.0		Karnofsky (6)

(Continued)

Table 1 (Cont'd)

Agent	Species[a]	No. of Patients or Animals	Drug Administration Route	Drug Administration Schedule (days)	Period of Obs. of Animals for Toxicity (in Days; or Median Days to Max. Toxicity in Man	Toxicologic End Point for Species Indicated	Brief Toxicity "Rating" in Man; and Intensity of Major Reactions in Large Animals Rating[b] 0	Mild	Mod.	Severe	Limiting Toxicologic Symptoms or Reactions	Daily Dose (mg/kg)	Daily Dose "Corrected" to qd 1-5 day Schedule (mg/kg)	"Corrected" Dosage Level (qd 1-5 days) Converted to Surface Area Basis[c] km Factor	mg/m²	Ratio (mg/m²) (Animal/Man)	Reference
4. 5-FUDR (Cont'd)	Swiss mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						150.0	150.0	3.0	450.0	0.41	Schmidt (7)
	BDF_1 mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						150.0	150.0	3.0	450.0	0.41	Schmidt (7)
	Swiss mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						126.0	179.0	3.0	537.0	0.48	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						105.0	147.0	3.0	441.0	0.40	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-11	1-21	LD_{10}						126.0	277.0	3.0	831.0	0.75	Griswold (3)
	Hamster	50-100	I. P.	qd 1-7	1-14	LD_{10}						39.0	55.0	3.0	165.0	0.15	Griswold (8)
	H. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						88.0	88.0	5.2	458.0	0.41	Schmidt (7)
	F. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						90.0	90.0	5.2	468.0	0.42	Schmidt (7)
	Dog	6	I. V.	qd 1-10	1-40	MTD						20.0	40.0	19.0	760.0	0.68	Philips (37)
	Monkey	6	I. V.	qd 1-6	1-60	MTD		MD; GI				50.0	60.0	11.5	690.0	0.62	Ratt (9)
5. Nitrogen mustard (HN2)	Man	15	I. V.	qd 1-5	15	MTD	0	0	15	0	MD; GI	0.2	0.2	37.0	7.4[e]		Clifford et al. (13)
		8	I. V.	Day 1 only	7	MTD	0	0	8	0	MD; GI	1.0	0.2	37.0	7.4		Kretchmer et al (14)
		Large no.	I. V.	Day 1 only		Usual dose					MD; GI	0.4	0.08	37.0	3.0		Karnofsky (6)
	Swiss mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						1.5	1.5	3.0	4.5	0.61	Schmidt (7)
	BDF_1 mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						1.5	1.5	3.0	4.5	0.61	Schmidt (7)
	Swiss mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						0.68	1.0	3.0	3.0	0.41	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						0.33	0.46	3.0	1.4	0.19	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-11	1-21	LD_{10}						0.30	0.66	3.0	2.0	0.27	Griswold (3)
	Hamster	50-100	I. P.	qd 1-7	1-14	LD_{10}						0.90	1.3	4.1	5.3	0.72	Griswold (8)
	H. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						0.48	0.48	5.2	2.5	0.34	Schmidt (7)
	F. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						0.25	0.25	5.2	1.3	0.18	Schmidt (7)
	Dog	4	I. V.	qd 1-12 to 16	1-17	MTD		GI	MD			0.17	0.48	19.0	9.1	1.2	Schmidt (15)
	Monkey	15	I. V.	qd 1-8 to 16	1-19	MTD		MD; GI				0.084	0.20	11.5	2.3	0.31	Schmidt (15)
6. Nitromin	Man	71	I. V.	qd 1-5	21	MTD	40%		55%	5%	MD; CNS	2.0	2.0	37.0	74.0[e]		Cluse (18)
	Swiss mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						52.0	52.0	3.0	156.0	2.1	Schmidt (7)
	BDF_1 mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						46.0	46.0	3.0	138.0	1.9	Schmidt (7)
	Swiss mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						27.0	38.0	3.0	114.0	1.5	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						20.0	28.0	3.0	84.0	1.1	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-11	1-21	LD_{10}						9.0	20.0	3.0	60.0	0.81	Griswold (3)
	H. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						8.6	8.6	5.2	45.0	0.61	Schmidt (7)
	F. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						5.4	5.4	5.2	28.0	0.38	Schmidt (7)
	Dog	4	I. V.	qd 1-8 to 15	1-16	MTD	GI		MD		MD; tremors; convulsions	2.1	4.4	19.0	84.0	1.1	Schmidt (15)
	Monkey	8	I. V.	qd 1-8 to 15	1-16	MTD	GI		MD			2.1	4.8	11.5	55.0	0.74	Schmidt (15)
7. L-Phenylalanine mustard	Man	210	I. V.	Single dose	10-12	MTD	10%	10%	60%	20%	MD	1.0	0.2	37.0	7.4[e]		Burns et al. (17, 18)
		70	P.O.	qd 1-4	10-12	MTD	10%	10%	60%	20%	MD	0.2	0.16	37.0	5.9		Burns et al. (17, 18)
	Swiss mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						5.1	5.1	3.0	15.3	2.1	Schmidt (7)
	BDF_1 mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						5.5	5.5	3.0	16.5	2.2	Schmidt (7)
	H. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						2.8	2.8	5.2	14.6	2.0	Schmidt (7)
	F. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						1.7	1.7	5.2	8.8	1.2	Schmidt (7)
	Dog	10	I. V.	qd 1-12 to 16	1-19	MTD			GI	MD		0.21	0.63	19.0	12.0	1.6	Schmidt (15)
	Monkey	9	I. V.	qd 1-9 to 17	1-18	MTD		GI	MD			0.31	0.55	11.5	6.3	0.85	Schmidt (15)
8. Alanine mustard	Man	34	I. V.	qd 1-5	14-21	MTD	50%	30%	20%	0%	MD	0.9	0.9	37.0	33.0		Dietrich et al. (19)
	Swiss mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						1.5	6.3	3.0	18.9	0.57	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						7.2	10.0	3.0	30.0	0.91	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-11	1-21	LD_{10}						4.2	9.3	3.0	27.9	0.85	Griswold (3)
	Hamster	50-100	I. P.	qd 1-7	1-14	LD_{10}						8.0	11.2	4.1	46.0	1.4	Griswold (8)
	Dog	4	I. V.	qd 1-8 to 15	1-16	MTD		GI	MD			0.63	1.5	19.0	29.0	0.88	Schmidt (15)
	Monkey	5	I. V.	qd 1-8 to 16	1-17	MTD				MD		0.63	1.5	11.5	17.0	0.52	Schmidt (15)

(Continued)

Table 1 (Cont'd)

Agent	Species[a]	No. of Patients or Animals	Drug Administration: Route	Drug Administration: Schedule (days)	Period of Obs. of Animals for Toxicity in Days; or Median Days to Max. Toxicity in Man	Toxicologic End Point for Species Indicated	Brief Toxicity "Rating" in Man; and Intensity of Major Reactions in Large Animals: Rating[b] 0	Mild	Mod.	Severe	Limiting Toxicologic Symptoms or Reactions	Daily Dose (mg/kg)	Daily Dose "Corrected" to qd 1-5 day Schedule (mg/kg)	"Corrected" Dosage Level (qd 1-5 days) Converted to Surface Area Basis[c]: km Factor	mg/m²	Ratio (mg/m²) (Animal / Man)	Reference
9. Cytoxan	Man	30	I. V.	Single dose	7-10	MTD	0%	50%	35%	15%	MD; GI	30.0	10.0	37.0	370.0[a]		Coggins et al. (20)
		27	I. V.	qd 1-6	7-10	<MTD	20%	55%	20%	5%		7.5	9.0	37.0	333.0		Coggins et al. (20)
			I. V.	qd 1-5		Usual dose						10.0	10.0	37.0	370.0		Karnofsky (6)
	Swiss mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						160.0	160.0	3.0	480.0	1.30	Schmidt (7)
	BDF_1 mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						130.0	130.0	3.0	390.0	1.06	Schmidt (7)
	Swiss mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						50.0	70.0	3.0	210.0	0.57	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						61.0	85.0	3.0	255.0	0.69	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-11	1-21	LD_{10}						57.0	125.0	3.0	375.0	1.01	Griswold (3)
	Swiss mouse	50-100	I. P.	Single dose	1-30	LD_{10}						252.0	50.0	3.0	150.0	0.41	Schabel (21)
	Hamster	50-100	I. P.	qd 1-7	1-14	LD_{10}						56.0	78.0	4.1	320.0	0.86	Griswold (8)
	H. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						14.0	14.0	5.2	73.0	0.20	Schmidt (7)
	F. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						10.3	10.3	5.2	54.0	0.15	Schmidt (7)
	Dog	7	I. V.	qd 1-7 to 15	1-18	MTD	GI		MD			5.6	12.3	19.0	234.0	0.63	Schmidt (15)
	Monkey	12	I. V.	qd 1-8 to 15	1-16	MTD		GI		MD		22.4	64.0	11.5	821.0	1.66	Schmidt (15)
10. ThioTEPA	Man	87	I. V.	qd 1-5	15	MTD	30%	30%	25%	15%	MD	0.2	0.2	37.0	7.4[a]		Moore (22)
			I. V.	qd 1-5		Usual dose						0.2	0.2	37.0	7.4		Karnofsky (6)
	Swiss mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						6.2	6.2	3.0	18.6	2.5	Schmidt (7)
	BDF_1 mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						6.2	6.2	3.0	18.6	2.5	Schmidt (7)
	Swiss mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						3.7	5.2	3.0	15.6	2.1	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						5.8	8.1	3.0	24.3	3.3	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-11	1-21	LD_{10}						2.4	5.3	3.0	15.9	2.1	Griswold (3)
	Hamster	50-100	I. P.	qd 1-7	1-14	LD_{10}						7.3	10.2	4.1	41.8	5.6	Griswold (8)
	H. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						3.0	3.0	5.2	15.6	2.1	Schmidt (7)
	F. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						2.3	2.3	5.2	12.0	1.6	Schmidt (7)
	Dog	5	I. V.	qd 1-10 to 17	1-18	MTD	GI		MD			0.38	1.1	19.0	20.9	2.8	Schmidt (15)
	Monkey	6	I. V.	qd 1-8 to 17	1-18	MTD	GI		MD			0.38	1.0	11.5	11.5	1.6	Schmidt (15)
11. Myleran	Man	13	Oral	qd 1-4[h]	16	MTD	0%	40%	30%	30%	MD	0.8	0.7	37.0	25.0[a]		Sullivan (31)
			Oral	qd 1-30		Usual dose						0.1	0.6	37.0	22.2		Karnofsky (6)
	Swiss mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						15.0	15.0	3.0	45.0	1.8	Schmidt (7)
	BDF_1 mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						15.0	15.0	3.0	45.0	1.8	Schmidt (7)
	H. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						4.1	4.1	5.2	21.0	0.84	Schmidt (7)
	F. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						3.2	3.2	5.2	17.0	0.68	Schmidt (7)
	Dog	7	I. V	qd 1-14 to 15	1-16	MTD		MD				2.0	6.0	19.0	114.0	4.8	Schmidt (15)
	Monkey	14	I. V.	qd 1-14 to 18	1-17	MTD	GI	MD				2.0	6.0	11.5	69.0	2.8	Schmidt (15)
12. BCNU	Man	7	I. V.	qd 1-3	42	MTD	0	4	2	1	MD	4.1	2.5	37.0	93.0[a]		DeVita et al. (23)
		8	I. V.	Single dose	44	MTD	0	4	4	0	MD	6.1	1.8	37.0	69.0[aa]		DeVita et al. (23)
	Swiss and BDF_1	50-100	I. P.	qd 1-5	1-24	LD_{10}	Note: BCNU possesses an unusual propensity to cause delayed toxicity even at extended periods after cessation of drug administration (in small animals, large animals, and man).					13.0	13.0	3.0	39.0	0.42	Schmidt (15)
	Swiss and BDF_1	50-100	Oral	qd 1-5	1-24 to 33	LD_{10}						11.0	11.0	3.0	33.0	0.35	Schmidt (15)
	Swiss mouse	50-100	I. P.	Day 1 only	1-30	LD_{10}						10.0	3.8	3.0	11.4[aa]	0.19	Schabel (21)
	Swiss mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						12.0	16.8	3.0	50.4	0.54	Schabel (21)
	BDF_1 mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						14.0	19.6	3.0	58.8	0.63	Schabel (21)
	Swiss mouse	50-100	Oral	qd 1-7	1-14	LD_{10}						8.4	11.8	3.0	35.4	0.38	Schabel (21)
	BDF_1 mouse	50-100	Oral	qd 1-7	1-14	LD_{10}						12.0	16.8	3.0	50.4	0.54	Schabel (21)
	BDF_1 mouse	50-100	I. P.	qd 1-11	1-21	LD_{10}						8.1	17.8	3.0	53.4	0.57	Schabel (21)
	BDF_1 mouse	50-100	Oral	qd 1-11	1-21	LD_{10}						7.5	16.5	3.0	49.5	0.53	Schabel (21)
	Hamster	50-100	I. P.	qd 1-7	1-14	LD_{10}						8.3	11.6	4.1	47.6	0.61	Schabel (21)
	Rat	50-100	I. P.	qd 1-5	1-25	LD_{10}						6.6	6.6	5.2	34.3	0.37	Schmidt (15)
	Dog	15	I. V.	qd 1-4 to 17	1-18	>MTD (3/6 deaths)			GI	MD		<1.25	<2.75	19.0	<52.3	<0.56	Schmidt (15)
	Dog	13	Oral	qd 1-8 to 19	1-50	ca MTD (1/5 deaths)			GI	MD		1.25	2.0	19.0	38.0	0.41	Schmidt (15)
	Monkey	15	I. V.	qd 1-5 to 16	1-17	MTD	GI —	GI	MD —	MD		2.5	5.0	11.5	58.0	0.62	Schmidt (15)
	Monkey	15	Oral	qd 1-7 to 15	1-29	MTD	GI	MD				2.5	5.5	11.6	63.3	0.68	Schmidt (15)

(Continued)

Table 1 (Cont'd)

Agent	Species[a]	No. of Patients or Animals	Drug Administration: Route	Drug Administration: Schedule (days)	Period of Obs. of Animals for Toxicity in Days; or Median Days to Max. Toxicity in Man	Toxicologic End Point for Species Indicated	Brief Toxicity "Rating" in Man; and Intensity of Major Reactions in Large Animals Rating[b]: 0	Mild	Mod.	Severe	Limiting Toxicologic Symptoms or Reactions	Daily Dose (mg/kg)	Daily Dose "Corrected" to qd 1-5 day Schedule (mg/kg)	"Corrected" Dosage Level (qd 1-5 days) Converted to Surface Area Basis[c]: km Factor	mg/m²	Ratio (mg/m²)/(Animal / Man)	Reference
13. Actinomycin D	Man	25	I. V.	qd 1-5	12	MTD	0%	55%	30%	15%	GI; MD	0.015	0.015	37.0	0.55[d]		Moore et al. (24)
			I. V.	qd 1-5		Usual dose					GI; MD	0.015	0.015	37.0	0.55		Karnofsky (6)
	Swiss mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						ca 0.08	ca 0.08	3.0	ca 0.18	ca 0.33	Schmidt (7)
	BDF_1 mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						0.11	0.11	3.0	0.33	0.60	Schmidt (7)
	Swiss mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						0.08	0.08	3.0	0.24	0.44	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						0.096	0.13	3.0	0.39	0.71	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-11	1-21	LD_{10}						0.05	0.11	3.0	0.33	0.60	Griswold (3)
	Hamster	50-100	I. P.	qd 1-7	1-14	LD_{10}						0.044	0.06	4.1	0.25	0.45	Griswold (8)
	H. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						0.08	0.08	5.2	0.42	0.76	Schmidt (7)
	F. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						0.09	0.09	5.2	0.47	0.85	Schmidt (7)
	Dog		I. V.	qd 1-15		MTD						0.01	0.03	19.0	0.57	1.0	Philips et al. (25)
14. Mitomycin C	Man	12	I. V.	qd 1-4	21	MTD	1	1	7	3	MD	0.25	0.20	37.0	7.4[e]		Miller et al. (26)
		50	I. V.	qd 1-10[h]	15	MTD	10%	40%	45%	5%	MD	0.10	0.20	37.0	7.4		Evans (27)
			I. V.	qd 1-10		Usual dose						0.10	0.20	37.0	7.4		Karnofsky (6)
	Swiss mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						1.7	1.7	3.0	5.1	0.69	Schmidt (7)
	BDF_1 mouse	50-100	I. P.	qd 1-5	1-21	LD_{10}						1.8	1.8	3.0	5.4	0.73	Schmidt (7)
	Swiss mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						2.1	2.9	3.0	8.7	1.2	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						2.1	2.9	3.0	8.7	1.2	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-11	1-21	LD_{10}						0.8	1.8	3.0	5.4	0.73	Griswold (3)
	Hamster	50-100	I. P.	qd 1-7	1-14	LD_{10}						1.2	1.7	4.1	7.0	0.95	Griswold (8)
	H. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						1.3	1.3	5.2	6.8	0.93	Schmidt (7)
	F. Rat	50-100	I. P.	qd 1-5	1-21	LD_{10}						ca 1.2	ca 1.2	5.2	ca 6.2	ca 0.84	Schmidt (7)
	Monkey	6	I. V.	qd 1-2	1-30	MTD		GI	MD			1.6	0.64	11.6	7.4	1.0	Rall (9)
15. Vinblastin	Man	20	I. V.	qd 1-3	10-14	MTD	0%	10%	40%	50%	MD	0.13	0.06	37.0	3.0[e]		Hertz et al. (28)
		10	I. V.	qd 1-3	10-14	<MTD	0	4	2	4	MD	0.10	0.06	37.0	2.2		Goldenberg (29)
		21	I. V.	qd 1-5 (2-7)	10-14	>MTD	0	6	7	8 (deaths)	MD	0.10	0.10	37.0	3.7		Smart et al. (30)
			I. V.	once wk x 5		Usual dose					MD	0.16	(0.05)	37.0	(1.1)		Karnofsky (6)
	Swiss mouse	50-100	I. P.	Single dose	1-30	LD_{10}						2.7	0.54	3.0	1.6	0.53	Schabel (21)
	Swiss mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						0.48	0.67	3.0	2.0	0.67	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-11	1-21	LD_{10}						0.24	0.53	3.0	1.6	0.53	Griswold (3)
	Hamster	50-100	I. P.	qd 1-7	1-14	LD_{10}						0.38	0.53	4.1	2.2	0.73	Griswold (8)
16. Vincristine	Man	28	I. V.	Single dose	14-21	MTD	0%	10%	50%	40%	Periph. nerve and GI	0.1	0.02	37.0	0.74		Carbone et al. (32)
		10	I. V.	qd 1-4[i]	14-21	MTD	50%		40%	10%	Periph. nerve and GI	0.03	0.024	37.0	0.89[e]		Carey et al. (33)
			I. V.	once wk x 4		Usual dose					MD	0.05	(0.01)	37.0	(0.37)		Karnofsky (6)
	Swiss mouse	50-100	I. P.	Single dose	1-30	LD_{10}						1.1	0.22	3.0	0.66	0.74	Schabel (21)
	Swiss mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						0.1	0.14	3.0	0.42	0.47	Schabel (21)
	BDF_1 mouse	50-100	I. P.	qd 1-11	1-21	LD_{10}						0.09	0.20	3.0	0.60	0.67	Schabel (21)
	Hamster	50-100	I. P.	qd 1-7	1-14	LD_{10}						0.24	0.34	4.1	1.4	1.6	Schabel (21)
17. Methyl GAG	Man	36	I. V.	qd 1-14[h]	10-21	MTD	(5%)	(20%)	(45%)	(30%)	GI; skin	4.1	11.4	37.0	420.0[e]		Levin et al. (34)
		13	I. V.	qd 1-11[h]	10-14	MTD	(5%)	(30%)	(45%)	(30%)	GI; skin	5.0	11.0	37.0	410.0		Levin et al. (34)
	Swiss mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						42.0	58.8	3.0	176.0	0.42	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-7	1-14	LD_{10}						61.0	85.4	3.0	256.0	0.61	Griswold (3)
	BDF_1 mouse	50-100	I. P.	qd 1-11	1-21	LD_{10}						46.0	101.2	3.0	304.0	0.72	Griswold (3)
	Hamster	50-100	I. P.	qd 1-7	1-14	LD_{10}						29.0	41.0	4.1	168.0	0.40	Griswold (8)
18. Hydroxyurea	Man	36	Oral	qd 1-11[h]	3-28	<MTD	0%	(10%)	(80%)	(10%)	MD	80.0	132.0	37.0	4900.0		Thurman et al. (35)
		19	Oral	qd 1-10[h]	3-28	MTD	0%	0%	(50%)	(50%)	MD	80.0	160.0	37.0	5900.0[e]		Thurman et al. (35)
	Dog	12	I. V.	qd 1-28	1-56	MTD			MD			100.0	560.0	19.0	10640.0	1.8	Rall (9)

(Continued)

Table 1 (Cont'd)

Note. (a) All of the human toxicity data are calculated on the basis of a 60-kg man (km factor = 37); approximately 20-gram mice were employed; 50-gram hamsters; 100-gram rats (except where otherwise indicated); 2.5-kg Rhesus monkeys; 7 to 8-kg young Beagle dogs (7-12 months of age).

(b) Numbers of patients exhibiting the indicated degree of "toxicity" are given unless the value is indicated as per cent. The intensity of marrow depression and gastrointestinal toxicity listed is the average or most frequent observed for dogs or monkeys receiving the dosage indicated.

(c) The human dosage (qd 1-5 day, mg/m^2) indicated by an asterisk was used to obtain the animal : man ratios. Underlined values represent studies in which mg/m^2 was the original basis for dosage.

(d) H. rat is the Holtzman line of Sprague-Dawley rat; F. rat is Fischer rat; S. D. is Sprague-Dawley rat.

(e) Average patient received one additional half dose on day 7. Maximum of 11 half doses given q. o. d.

(f) Average patient received four additional half doses q. o. d. Maximum of 11 half doses given q. o. d.

(g) Average patient received no additional therapy.

(h) Median duration of therapy to toxicity for daily treatment.

(i) Four additional half doses on days 7, 9, 11, and 13.

Table 2

A Comparison of Small-Animal LD_{10}'s, Large-Animal Maximum Tolerated Doses, and Human Maximum Tolerated Doses on a Mg/Kg Basis

		Mg/Kg (qd 1-5 Day Schedule)					
	Agent	LD_{10}: Swiss Mouse	LD_{10}: BDF_1 Mouse	LD_{10}: Rat	MTD: Rhesus Monkey	MTD: Dog	MTD: Man
1.	Amethopterin	3.2	5.2	0.58	3.0	0.12	0.41
2.	6-Mercaptopurine	86.0	62.0	51.0	56.0	22.0	27.0
3.	5-Fluorouracil	42.0	45.0	25.0	18.0	10.0	15.0
4.	5-FUDR	160.0	190.0	89.0	59.0	40.0	30.0
5.	Nitrogen Mustard	1.3	0.90	0.37	0.2	0.48	0.2
6.	Nitromin	45.0	31.0	7.10	4.8	4.4	2.0
7.	L-Phenylalanine	5.1	5.5	2.3	0.55	0.63	0.2
8.	Alanine Mustard	6.3	9.7	-	1.5	1.5	0.9
9.	Cytoxan	93.0	110.0	12.0	54.0	12.0	10.0
10.	ThioTEPA	5.7	6.5	2.7	1.0	1.1	0.2
11.	Myleran	15.0	15.0	3.7	6.0	6.0	0.7
12.	BCNU	11.0	16.0	6.6	5.3	2.4	2.5
13.	Actinomycin D	0.07	0.12	0.09	-	0.03	0.015
14.	Mitomycin C	2.3	2.2	1.3	0.64	-	0.2
15.	Vinblastin	0.60	0.53	-	-	-	0.08
16.	Vincristine	0.18	0.20	-	-	-	0.024
17.	Methyl GAG	59.0	93.0	-	-	-	11.0
18.	Hydroxyurea	-	-	-	-	560.0	160.0

Note: Average animal doses have been compared with human doses indicated by an asterisk in Table 1, and have been rounded to two significant figures.

Table 3

A Comparison of Small-Animal LD_{10}'s, Large-Animal Maximum Tolerated Doses and Human Maximum Tolerated Doses on a mg/m^2 Basis

	Agent	LD_{10} Swiss Mouse (km=3)	LD_{10} BDF_1 Mouse (km=3)	LD_{10} Hamster (km=4.1)	LD_{10} Rat (km=5.2)	MTD Rhesus Monkey (km=11.5)	MTD Dog (km=19)	MTD Man (km=37)	Estimated MTD Man (All Systems)
1.	Amethopterin	9.5	16.0	103.0	3.1	35.0	2.0	15.0	11.6
2.	6-Mercaptopurine	257.0	186.0	320.0	266.0	644.0	434.0	1000.0	327.0
3.	5-Fluorouracil	126.0	135.0	70.0	130.0	207.0	190.0	555.0	154.0
4.	5-FUDR	494.0	574.0	165.0	463.0	690.0	760.0	1110.0	514.0
5.	Nitrogen Mustard	3.8	2.6	5.3	1.9	2.3	9.1	7.4	3.1
6.	Nitromin	135.0	94.0		37.0	55.0	84.0	74.0	73.0
7.	L-Phenylalanine Mustard	15.0	17.0		12.0	6.3	12.0	7.4	11.5
8.	Alanine Mustard	19.0	29.0	46.0		17.0	29.0	33.0	22.8
9.	Cytoxan	280.0	340.0	320.0	64.0	621.0	234.0	370.0	266.0
10.	ThioTEPA	17.0	20.0	42.0	14.0	11.5	21.0	7.4	16.5
11.	Myleran	45.0	45.0		19.0	69.0	114.0	25.0	47.4
12.	BCNU	34.0	47.0	48.0	34.0	61.0	45.0	93.0	43.8
13.	Actinomycin D	0.21	0.35	0.25	0.45		0.57	0.55	0.34
14.	Mitomycin C	6.9	6.5	7.0	6.5	7.4		7.4	6.9
15.	Vinblastin	1.8	1.6	2.2				3.0	1.8
16.	Vincristine	0.54	0.60	1.4				0.89	0.63
17.	Methyl GAG	176.0	280.0	168.0				420.0	211.0
18.	Hydroxyurea						10,640.0	5900.0	-

Note: Average animal doses have been compared with human doses indicated by an asterisk in Table 1. The last column is the weighed estimate from the animal results (See Appendix III).

Table 4. Various Estimated Values Assuming Model (1) and Model (2).

Model (1) (Dose in man mg/m^2) = 1 (Dose in animal system [mg/m^2])

Animal System	St. Deviation (log scale)	Multipliers for dose in animal system giving lower and upper standard deviation limits (mg/m^2 scale) lower*	upper*
1. monkey	.312	.49	2.1
2. Swiss mouse	.369	.43	2.3
3. BDF_1 mouse	.379	.42	2.4
4. dog	.422	.38	2.6
5. rat	.495	.32	3.1
6. hamster	.601	.25	4.0
all combined (weighted)	.299	.50	2.0

Model (2) (Dose in man mg/m^2) = A_i (Dose in animal system [mg/m^2]).

Animal System	Estimate of A_i	$A_i \pm 2$ S.E.	St. Deviation (log scale)	Multipliers for dose in animal system giving lower and upper st. deviation limits (mg/m^2 scale) lower	upper
1. monkey	1.15	.79 - 1.67	.293	.51	2.0
2. Swiss mouse	1.39	.93 - 2.06	.323	.48	2.1
3. rat	2.08	1.35 - 3.21	.339	.46	2.2
4. BDF_1 mouse	1.29	.84 - 1.97	.346	.45	2.2
5. dog	1.05	.60 - 1.83	.400	.40	2.5
6. hamster	1.32	.61 - 2.86	.556	.28	3.6
all combined (weighted)	1.36	1.13 - 1.60	.275	.53	1.9

*As an example, the toxic dosage of amethopterin in the monkey is 40.2 $mg./m^2$. Thus, the predicted MTD in man is 40.2 mg/m^2 with one st. deviation limits 40.2 x .49 = 19.7 mg/m^2 to 40.2 x 2.1 = 84.4 $mg./m^2$.

Table 5

Predicted Dosages (mg/m^2) in Man Using Each Animal System and All Systems Combined

	Agent	Swiss Mice	BDF Mice	Hamster	Rat	Monkey	Dog	Overall Unweighted	Overall Weighted	Man
1.	Amethopterin	13.2	20.6	116.0	6.4	40.2	2.5	17.0	15.7	15.0
2.	6-Mercaptopurine	357.0	240.0	424.0	554.0	740.0	457.0	435.0	444.0	1000.0
3.	5-Fluorouracil	244.0	174.0	92.7	271.0	238.0	200.0	182.0	210.0	555.0
4.	5-FUDR	686.0	740.0	219.0	964.0	793.0	800.0	581.0	699.0	1110.0
5.	Nitrogen Mustard	5.3	3.4	7.0	4.0	2.6	9.6	4.8	4.2	7.4
6.	Nitromin	187.0	121.0		77.0	63.1	88.3	99.1	99.6	74.0
7.	L-Phenylalanine Mustard	20.8	21.9		25.0	7.2	12.6	16.0	15.6	7.4
8.	Alanine Mustard	26.4	37.3	61.0		19.5	30.5	35.3	31.0	33.0
9.	Cytoxan	388.0	439.0	424.0	133.0	711.0	246.0	345.0	362.0	370.0
10.	ThioTEPA	23.6	25.8	55.6	29.1	13.2	22.1	25.7	22.5	7.4
11.	Myleran	62.4	58.0		39.5	79.3	120.0	66.8	64.6	25.0
12.	BCNU	47.2	60.5	63.5	70.8	68.4	47.3	59.1	59.6	93.0
13.	Actinomycin D	0.29	0.27	0.33	0.44		0.54	0.46	0.46	0.55
14.	Mitomycin C	9.6	8.4	9.3	13.5	8.5		9.2	9.3	7.4
15.	Vinblastin	2.5	2.1	2.9					2.4	3.0
16.	Vincristine	0.75	0.77	1.9					0.85	0.89
17.	Methyl GAG	244.0	361.0	222.0					287.0	420.0
18.	Hydroxyurea						11,190.0			5900.0

APPENDIX I

More Detailed Description of the Toxicologic Data Used

Small animals (mouse, rat, and hamster).—The classic end point for assessing drug toxicity to small animals is death (LD10, LD50, LD90). A reliable method of determining the lethality of a drug is to give an appropriately spaced series of doses to groups of about 10 animals each; to record percent deaths at each drug level; and then to plot the dose-mortality data on log-probit paper (7), draw a line of best fit, and read the lethal dose for 10, 50, or 90%, or any other fraction of the animals. The reliability of such end points depends on the number of animals, and the LD10, LD50, or LD90 (in mg/kg or mg/m^2) for a given animal species is incomplete unless it is accompanied by information on the route of administration, the dosage schedule, and the period of observation for delayed death after cessation of drug administration. Useful information may be gained from the median day of death, during and after administration of various dose levels, and the slope of the dose-mortality curve.

Most of the mouse toxicity data in this analysis were obtained by Schmidt (7) and Griswold et al. (3); the rat toxicity data by Schmidt (7); and the hamster toxicity data by Griswold et al. (8). All toxicity data were plotted as indicated previously and values were read from lines of best fit. About 50 to more than 100 animals were used in each toxicity determination. The ip route was used in most instances, and all animals were kept for 1–3 weeks after the end of treatment for observation of delayed death. The schedules used most frequently were qd 1–5, qd 1–7, qd 1–11, and qd 1–15 days.

We are aware that the LD10 is not as reliable statistically as the LD50; however the LD10 is closer to the maximum doses accepted in typical experimental cancer chemotherapy trials and to the maximum doses reached in clinical drug evaluation.

Some indication of the overall reproducibility and reliability of LD10's obtained by the general procedure described may be found in calculations by Griswold et al. (3): "among the 219 LD10's determined (Swiss mice, qd 1–7; BDF$_1$ mice, qd 1–7 and qd 1–11 days), the median range between the lower and upper 95% confidence limits was 0.35 logs." No consistent difference was observed in the toxicity of a wide variety of agents to randombred Swiss mice and inbred BDF$_1$ mice (3).

The procedures for obtaining and interpreting toxicity data for the rat and hamster were essentially the same as those described for the mouse.

Large animals (dog and monkey).—Since it is rarely feasible to obtain extensive dose-mortality data for dogs and monkeys, accurate LD10's, LD50's, or LD90's usually are not available. However the lethal dose range in such species is determined for anticancer agents being considered for clinical trial. In general the dose-mortality data for dogs and monkeys consisted of daily dose levels (2-fold increases) given to groups of 2–4 animals up to 100% mortality. The approximate toxicologic end point selected for this analysis was the highest dose which killed 0% of 2–4 animals. Usually, doubling this dose killed all the animals. As with other species, the dose levels given to dogs and monkeys were corrected to a schedule of qd 1–5 days.

The major limiting toxic effects of the classes of agents considered in this analysis were marrow depression and gastrointestinal lesions. Table 1 (Appendix I) presents the basis for rating the intensity of these dose-related hematopoietic effects and gastrointestinal and soft tissue lesions.

Man.—Most clinical cancer chemotherapy studies use an experimental design in which the drug dose and schedule are varied so that each patient receives the optimum dose of the agent and therefore each patient becomes a unit of study. For this type of study, any analysis of the toxic effect of a certain dose, schedule, and route of administration becomes very difficult. For this reason the published literature and unpublished data available were searched for studies using a fixed-dose schedule and fixed route of administration for a series of patients, followed by a period of observation without chemotherapy. In such circumstances it was possible to assess the effects of treatment on the individual. When possible, studies were chosen of patients who had normal peripheral blood and bone marrow and who had not received marrow-suppressive therapy for the 6 weeks preceding the study. Another criterion for selecting data was that objective toxic effects were observed in a significant

Table 1. Appendix I

Rating of the Infensity of the Major Toxicologic Reactions as Observed in Dogs and Monkeys

Reaction		Basis for Rating as:			
Classification	Determined By	0	Mild (or +)	Moderate (or ++)	Severe (or +++)
Anemia*	Decrease in RBC count	Essentially none	1.0-1.5 x 10^6/cmm < control	<4.5 to >3.5 x 10^6/cmm	<3.5 x 10^6/cmm
Reticulocytopenia*	Decrease in retic-% RBC	Essentially none	>0.5%; <1/2 control	>0.01%; <0.05%	<0.01%
Hemoconcentration*	Increase in hematocrit	Essentially none	>10%; <20% control	>20%; <30% control	>30% control
Leucopenia*	Decrease in WBC count	Essentially none	<1/2 control	>2.5 x 10^3/cmm; <5 x 10^3/cmm	<2.5 x 10^3/cmm
Thrombocytopenia*	Decrease in platelet count	Essentially none	>10^5/cmm; <1/2 control	>10^4/cmm; <10^5/cmm	<10^4/cmm
Marrow depression*	Decrease in absolute count	Essentially none	>10^3/cmm; <5 x 10^5/cmm	>5 x 10^4/cmm; <10^5/cmm	<5 x 10^4/cmm
Hemorrhagic lesions**	GI tract	Essentially none	Isolated, punctate	Gross - limited area	Gross - widespread
Hemorrhagic lesions**	Generalized, soft tissue	Essentially none	Isolated, punctate	Gross - limited area	Gross - widespread
CNS stimulation	Convulsions	Essentially none		Described as observed	
Other	--	Essentially none		Described as observed	

Note: *Grouped under the term "marrow depression" (MD) in this general paper.

**Grouped under the term "gastrointestinal tract damage" (GI) in this paper.

Detailed data regarding specific hematologic and tissue and organ damage are available but are not included herein. In Table 1 of the text, only the average degree of marrow depression and gastrointestinal damage are presented under 0, mild, moderate, or severe.

number of patients treated with a certain dose and schedule. The most commonly used parameter was white blood cell count (WBC). The toxic manifestations were then graded on a 0 to 3+ scale, ie, none, mild, moderate, or severe (when possible). Chemotherapy experiments which used very small doses of drug given in periods of 6–8 weeks were not included because of the lack of an appropriate counterpart in experimental systems. Therefore we tried to find tests in which maximum tolerated doses were given in minimum time intervals by fixed-dose schedules (and fixed routes).

APPENDIX II

Relationship Between Drug Doses in Milligram Per Kilogram and in Milligram Per Square Meter of Surface Area for Man and for Small and Large Animals

In table 1 (Appendix II) the estimated square meters of surface area are given for several body weights (kg) within each mammalian species. The surface area in square meters was estimated by the formula

$$(\text{body surface area}) = \frac{K \times w^{2/3}}{10^4}.$$

The K values are given for each species by Spector (ref. 40, p 175) and w is body weight in grams. The K values differ among species and also within species; however a single K factor was chosen for each species except man. The conversion factors (km) were obtained simply by dividing the body weight by the surface area. Thus to convert a dose in mg/kg to a dose in mg/m², we use the approximate formula

$$(\text{dose in mg/m}^2) = (km) \times (\text{dose in mg/kg})$$

where the (km) factor is selected according to the species and body weight. For example, a dose of 20 mg/kg/day given to a 20-g mouse is approximately equal to $20 \times 3 = 60$ mg/m²/day.

Note that the (km) factor is simply

$$(km) = \frac{10^2 \times (\text{kg})^{1/3}}{K}$$

where kg is weight in kilograms. The (km) factors used in this study were

Species	Approx. wt. (kg)	(km) factor
Man	60	37
Mouse	.020	3.0
Rat	.100	5.2*
Hamster	.050	4.1
Monkey	2.5	11.5
Dog	7.0–8.0	19.0–19.8

* Except as otherwise indicated in table 1 of text.

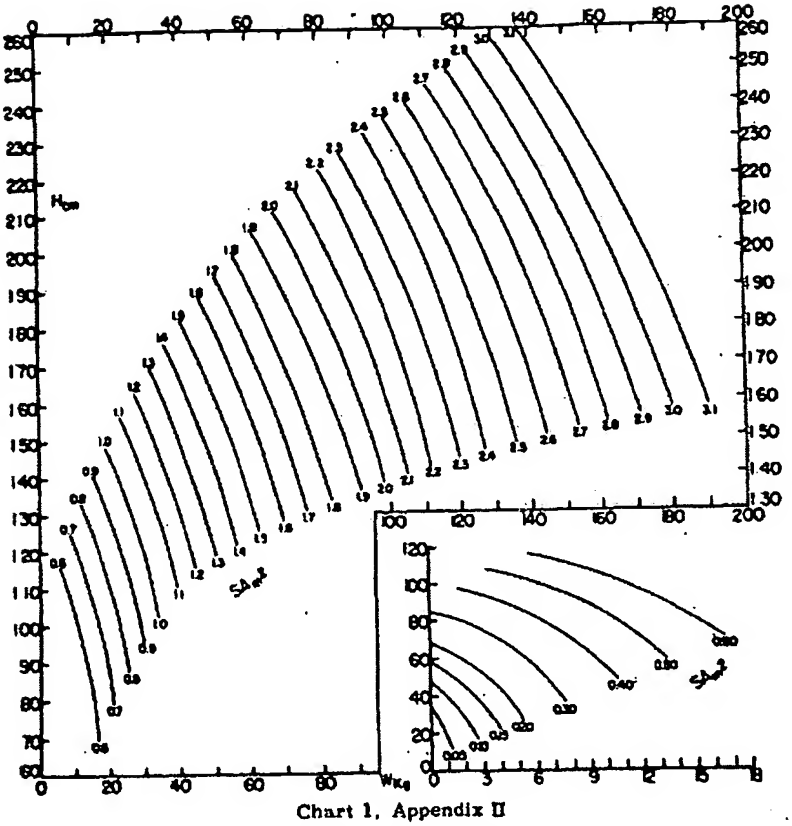


Chart 1, Appendix II

Diagram for Determination of Human Surface Area from Height and Weight. (Insert is Used for Low Range of SAm^2 from 0.05 to 0.60). Taken from Sendroy and Cecchini, J. Applied Physiol. 7: 1-12 (1954). H_{cm} = height in centimeters; W_{kg} = weight in kilograms; SAm^2 = surface area in sq. meters.

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Table I. Appendix II

Conversion Factors (Dosages in mg./kg to mg./m^2) for the Mouse, Rat, Monkey, Dog and Man Given Body Weight Only.

Species	K	Body Wt. (kg)	Square Meters Area	Conversion Factor (km)
Mouse	9.0	0.018	0.0062	2.9
		0.020	0.0066	3.0
		0.022	0.0071	3.1
		0.024	0.0075	3.2
Rat	9.0	.050	0.0122	4.1
		.070	0.0153	4.6
		.080	0.0167	4.8
		0.100	0.0194	5.2
		0.150	0.0254	5.9
		0.200	0.0308	6.5
		0.250	0.0357	7.0
Monkey	11.8	2.0	0.188	10.6
		2.5	0.217	11.5
		3.0	0.244	12.3
Dog	10.1	6.0	0.334	18.0
		7.0	0.369	19.0
		8.0	0.404	19.8
		9.0	0.437	20.6
Man (avg.)		5.0	0.26	19.0
		10.0	0.44	23.0
		20.0	0.80	25.0
		40.0	1.30	31.0
		60.0	1.62	37.0
		70.0	1.80	39.0
		80.0	1.96	41.0

Table 2, Appendix II

Table 2, Appendix II

Conversion Factors (Dosages in Mg/Kg to Mg/M^2 Body Surface Area) for Man Given Height and Body Weight

Body Wt.		Height																		
Kg	Pounds	Feet:	1.3	1.7	2.0	2.3	2.7	2.9	3.3	3.6	3.9	4.3	4.6	4.9	5.3	5.6	5.9	6.3	6.6	6.9	7.3
		Inches:	16	20	24	28	32	35	39	43	47	51	55	59	63	67	71	75	79	83	87
		Cm:	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220
5	11		22	19	17	15	14														
10	22		28	26	24	23	21	19	18												
15	33					26	25	24	22	21	19	18									
20	44						29	28	26	25	24	22	21								
25	55								30	28	27	26	24	23	22						
30	66								33	31	30	29	27	26	25						
35	77									34	32	31	30	28	27	26					
40	88										35	33	32	31	30	28	27				
45	99										37	35	34	33	31	30	29	28			
50	110										38	37	36	35	34	32	31	30	29		
55	121											39	38	37	35	34	33	32	31		
60	132											41	39	38	37	36	35	34	32	31	
65	143												41	40	39	38	36	35	34	33	
70	154												42	41	40	39	39	37	36	35	34
75	165												44	43	41	40	39	38	37	36	35
80	176												45	44	43	42	41	40	39	38	37
85	187													45	44	43	42	41	40	39	38
90	198														45	44	43	42	41	40	39
95	209														46	45	44	43	42	41	40
100	220															46	45	44	43	42	41
105	231															47	46	45	44	44	43
110	242															48	47	47	46	45	43

Note: The underlined conversion factors are for individuals of approximately average height to body weight ratios.

The above km factors were calculated from data presented in: Spector, W. S., Handbook of Biological Data, W. B. Saunders Company, Philadelphia and London (1956). The basic data (Spector) were derived according to the method of Sendroy and Cecchini, 1954 (Sendroy, J., Jr., and Cecchini, L. P., J. Applied Physiology 7: 1-12, 1954).

Example: A dosage of 2.5 mg/kg/day of 6-MP (to a 20-kilo child of 110-cm height) is equal to 2.5 x 25 (km factor) = 62.5 mg/m^2/day.

Table 2 (Appendix II) presents the (km) factors for man. Chart 1 (Appendix II) is a diagram for determining the surface area of humans from height and weight (taken from Sendroy and Cecchini [39]).

It may be of some interest to indicate how the results of the analysis would have changed if surface area had been estimated as

$$(\text{surface area}) = (\text{kg})^{\frac{1}{3}}.$$

The rationale is that since body surface area is clearly not the target area of action of the drug but presumably is proportional to the true target area, it is sufficient to measure surface area in units proportional to the true target area. The surface area unit is simply the two-thirds power of weight, though it is not easy to vizualize this quantity. This leads to the formula

$$(\text{dose in mg/surface area}) = (km) \times (\text{dose in mg/kg})$$

where $(km) = (\text{kg})^{\frac{1}{3}}$ instead of $[(\text{kg})^{\frac{1}{3}} \times 10^2]/K$ as before. If the K factors were the same for each species, the analysis in the new surface area unit would be exactly the same as that given. Since the K factors do differ among species, ranging from 9.0–11.8, the results of a re-analysis would differ slightly from those given here but certainly not substantially. The most appropriate K factor for any drug would be that which makes the two-thirds power of weight for each species equal to the surface area where the drug acts. Since this information is not generally known, it matters little whether the K factors among species are assumed to be the same or to differ slightly.

APPENDIX III

Statistical Considerations

The notation used is as follows:

y = true log (dose in mg/m^2) in man

x_i = true log (dose in mg/m^2) in animal system i, $(i = 1, \ldots, 6)$.

The doses are the MTD in man and the LD10 in each animal system. Now, y and x_i are variables that have particular values when a drug is given according to a certain schedule and route of administration (assumed here to be qd 1–5 days and the ip or iv route with a few exceptions). Because of random error, and other factors, we do not observe y and x_i, but

$$y' = y + d_i \quad \text{(A1)}$$

$$x' = x_i + e_i \quad \text{(A2)}$$

where d_i and e_i are random variables. We assume that d_i and e_i are independently distributed with zero means and are independent of y and x_i. The primes indicate observed values of y and x_i.

We postulate that the underlying structural relationship (model) is

$$y = \alpha_i + x_i, \quad (i = 1, \ldots, 6) \quad \text{(A3)}$$

where $\alpha_i = \log A_i$ according to the notation in the text. In model (1), α_i is zero and in model (2) it is a parameter to be estimated. These are the simplest models that could be considered. Actually the more general relationship $y = \alpha_i + \beta_i x_i$ was also considered but since the estimates of β_i $(i = 1, \ldots, 6)$ were all near 1, only the simpler models given will be investigated further.

Substituting (A1) and (A2) into (A3), we have

$$y' - d_i = \alpha_i + x_i' - e_i$$

$$y' = \alpha_i + x_i' + (d_i - e_i)$$

where $(d_i - e_i)$ is a random variable with zero mean. We have n_i pairs (usually 17) of observations, (y_j', x_{ij}), $j = 1, \ldots, n_i$, and we wish to estimate the parameter α_i in model (2). Since each animal system provides an estimate of y, we will also be interested in a combined estimate of y.

The aim in estimating the parameter of the model is to predict a value of y (denoted by $\hat{y}$) for a given value of x'. The prediction equation is

$$\hat{y} = \hat{\alpha}_i + x_i' \quad \text{(A4)}$$

As Lindley (38) noted, x_i' is measured without error and standard least squares may be used for estimating α_i. Thus the estimate of α_i, denoted by $\hat{\alpha}_i$, is simply

$$\hat{\alpha}_i = \frac{\sum_j (y_j' - x_{ij}')}{n_i}, \quad (i = 1, 2, \ldots, 6).$$

The values of A_i given in the text are the antilogs of $\hat{\alpha}_i$.

To obtain an estimate based on results from all animal systems, we can simply average the values of $\hat{y}$ from the six animal systems or calculate a weighted average where the weight for each y is inversely proportional to its variance. The weighted combined estimate is

$$\hat{y}_{wc} = \frac{\sum_{i=1}^{6} w_i (\hat{\alpha}_i + x_i)}{\sum_{i=1}^{6} w_i}$$

where

$$w_i = \frac{1/s_i^2}{\sum_{i=1}^{6} 1/s_i^2}$$

and s_i^2 is the variability about $\hat{y}$. That is,

$$s_i^2 = \frac{\sum_{j=1}^{n_i} (\hat{y}_j - y_j)^2}{n_i - 1}, \quad (i = 1, \ldots, 6)$$

for model (2). For model (1) the divisor is n_i.

A sample of the calculations required is given for illustrative purposes, assuming that only two drugs, amethopterin and 6-mercaptopurine (6-MP), have been studied in Swiss mice and man. The data are in log (dose in mg/m²):

Drug	*Man* ($\hat{y}_i$)	*Swiss mice* (x_i)
Amethopterin	1.176	0.978
6-MP	3.000	2.410

For model (1) the predicted values of the dose in man are simply the doses observed in Swiss mice, namely, 9.5 mg/m² for amethopterin and 257.0 mg/m² for 6-MP. The standard deviation is

$$s_i = \sqrt{\frac{(1.176 - .978)^2 + (3.00 - 2.410)^2}{2}} = 0.440.$$

For model (2) we have

$$\hat{\alpha}_i = \frac{\Sigma y_j - \Sigma x_j}{2} = \frac{4.176 - 3.388}{2} = 0.394$$

and so $\hat{A}_i = 2.48$. The predicted values of $\hat{y}$ in man are

Drug	*Equation*	*Dose* (mg/m^2)
Amethopterin	$\hat{y}_1 = 0.394 + .978 = 1.372$	23.6
6-MP	$\hat{y}_2 = 0.394 + 2.410 = 2.804$	636.8

The standard deviation for model (2) is:

$$s_i = \sqrt{\frac{(1.372 - 1.176)^2 + (3.000 - 2.804)^2}{1}} = 0.277$$

and $1/s_i^2$ is the term in the numerator and the first term in the denominator of w_i. The standard error of α_i is

$$\text{SE of } \alpha_i = \frac{0.277}{\sqrt{2}} = 0.196.$$

LIST OF COMPOUNDS

Actinomycin D: NSC-3053.

Alanine mustard: NSC-17663; DL-alanine, *N,N*-bis(2-chloroethyl)-, hydrochloride.

Amethopterin: NSC-740; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.

BCNU: NSC-409962; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

Cytoxan: NSC-26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, hydrate.

5-Fluorouracil: NSC-19893.

5-FUDR: NSC-27640; uridine, 2′-deoxy-5-fluoro-.

Hydroxyurea: NSC-32065.

6-Mercaptopurine: NSC-755; purine-6-thiol, hydrate.

Methyl-GAG: NSC-32946; guanidine, 1,1′-[(methylethanediylidine)dinitrilo]di-, dihydrochloride, hydrate.

Mitomycin C: NSC-26980; carbamic acid, ester with 6-amino-1,1a,2,8,8a,8b-hexahydro-8-(hydroxymethyl)-8a-methoxy-5-methylazirino[2′,3′:3,4]pyrrolo[1,2-*a*]-indole-4,7-dione.

Myleran: NSC-750; 1,4-butanediol, dimethanesulfonate.

Nitrogen mustard (HN2): NSC-762; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride.

Nitromin: NSC-10107; diethylamine, 2,2′-dichloro-*N*-methyl-, *N*-oxide, compd. with hydrochloride (1:1).

L-Phenylalanine mustard: NSC-8806; L-alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, hydrochloride.

ThioTEPA: NSC-6396; phosphine sulfide, tris(1-aziridinyl)-.

Vinblastine: NSC-49842; vincaleukoblastine, sulfate, hydrate.

Vincristine: NSC-67674; leurocristine, sulfate.

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